

Counts 09/770,102

=> d his 1

(FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT  
11:43:19 ON 23 JUL 2003)

L43 61 S L39 OR L42

=> d que 143

L1 4447 SEA CRAIG R?/AU  
L2 2 SEA L1 AND MODIFICATION(3A) DEPENDENT  
L3 76666 SEA BINDING(A) ASSAY#  
L4 12973 SEA PROTEIN(A) MODIFICATION  
L5 587803 SEA PROTEIN(3A) (PHOSHORYLA? OR DEPHOSPHORYLAT? OR KINAS?)  
L6 2767 SEA L3 AND (L4 OR L5)  
L7 212084 SEA (INCREAS? OR REDUC? OR DECREAS?) (3A) (BIND? OR DISSOCIATION)  
  
L8 292 SEA L7 AND L6  
L9 236 SEA L8 NOT PY>2000  
L10 5 SEA L9 AND (PARTNER# OR PAIR#)  
L11 4416 SEA PROTEIN#(A) BINDING(A) ASSAY#  
L13 136650 SEA (TAG? OR LABEL? OR FLUOROPHORE#) (5A) (PROTEIN# OR PEPTIDE#  
OR POLYPEPTIDE#)  
L14 141 SEA L13 AND L11  
L15 6 SEA L14 AND (L4 OR L5)  
L16 108762 SEA (TAG? OR LABEL? OR FLUOROPHORE#) (3A) (PROTEIN# OR PEPTIDE#  
OR POLYPEPTIDE#)  
L17 95907 SEA L16 NOT DNA  
L18 5348 SEA L17 AND (L4 OR L5)  
L19 44 SEA L18 AND IMMUNOASSAY#  
L20 198065 SEA (PHOSHORYLA? OR DEPHOSPHORYLA? OR KINAS?) (3A) ACTIVIT?  
L21 181134 SEA (DETECT? OR MEASUR? OR ASSAY? OR MONITOR?) (3A) (BIND? OR  
DISSOCIATI?)  
L22 1763 SEA L20 AND L21  
L23 300 SEA L22 AND (TAG? OR LABEL? OR FLUOROPHORE#)  
L24 34 SEA L23 AND L7  
L26 34982 SEA (DETECT? OR MEASUR? OR ASSAY? OR MONITOR?) (3A) ENZYM?(A)  
ACTIVIT?  
L27 16478 SEA (DETECT? OR MEASUR? OR ASSAY? OR MONITOR?) (3A) PROTEIN#(A)  
BIND?  
L28 25 SEA L26 AND L27  
L29 1017 SEA L26 AND (L4 OR L5)  
L30 16 SEA L29 AND IMMUNOASSAY#  
L31 23233 SEA PROTEIN(3A) GLYCOSYLAT?  
L32 53 SEA L31 AND L26  
L33 4 SEA L32 AND (BIND? OR DISSOCIAT?)  
L34 324 SEA L26 AND GLYCOSYLAT?  
L35 3 SEA L34 AND IMMUNOASSAY#  
L37 69 SEA PROTEIN(3A) (MODIFY? OR MODIFICAT?) (3A) ENZYM?(3A) ACTIVIT?  
L38 16 SEA L37 AND BIND?  
L39 2 DUP REM L2 (0 DUPLICATES REMOVED)  
L40 153 SEA L10 OR L15 OR L19 OR L24 OR L28 OR L30 OR L33 OR L35 OR  
L38  
L41 96 SEA L40 NOT PY>2000  
L42 59 DUP REM L41 (37 DUPLICATES REMOVED)  
L43 61 SEA L39 OR L42

=> d ibib abs 143 1-61

L43 ANSWER 1 OF 61 MEDLINE on STN

Counts 09/770,102

ACCESSION NUMBER: 2001118790 MEDLINE  
DOCUMENT NUMBER: 20562748 PubMed ID: 11108967  
TITLE: Metastasis-associated protein Mts1 (S100A4) inhibits CK2-mediated phosphorylation and self-assembly of the heavy chain of nonmuscle myosin.  
AUTHOR: Krajjevska M; Bronstein I B; Scott D J; Tarabykina S;  
Fischer-Larsen M; Issinger O; Lukanidin E  
CORPORATE SOURCE: Department of Molecular Cancer Biology, Institute of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark.  
CONTRACT NUMBER: EY08123 (NEI)  
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (2000 Dec 20) 1498 (2-3) 252-63.  
PUB. COUNTRY: Journal code: 0217513. ISSN: 0006-3002.  
Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200102  
ENTRY DATE: Entered STN: 20010322  
Last Updated on STN: 20020420  
Entered Medline: 20010215  
AB A role for EF-hand calcium-binding protein Mts1 (S100A4) in the phosphorylation and the assembly of myosin filaments was studied. The nonmuscle myosin molecules form bipolar filaments, which interact with actin filaments to produce a contractile force. Phosphorylation of the myosin plays a regulatory role in the myosin assembly. In the presence of calcium, Mts1 binds at the C-terminal end of the myosin heavy chain close to the site of phosphorylation by protein kinase CK2 (Ser1944). In the present study, we have shown that interaction of Mts1 with the human platelet myosin or C-terminal fragment of the myosin heavy chain inhibits phosphorylation of the myosin heavy chain by protein kinase CK2 in vitro. Mts1 might also bind directly the beta subunit of protein kinase CK2, thereby modifying the enzyme activity. Our results indicate that myosin oligomers were disassembled in the presence of Mts1. The short C-terminal fragment of the myosin heavy chain was totally soluble in the presence of an equimolar amount of Mts1 at low ionic conditions (50 mM NaCl). Depolymerization was found to be calcium-dependent and could be blocked by EGTA. Our data suggest that Mts1 can increase myosin solubility and therefore suppress its assembly.

L43 ANSWER 2 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 2000179877 MEDLINE  
DOCUMENT NUMBER: 20179877 PubMed ID: 10713090  
TITLE: Association of fibroblast growth factor receptor 1 with the adaptor protein Grb14. Characterization of a new receptor binding partner.  
AUTHOR: Reilly J F; Mickey G; Maher P A  
CORPORATE SOURCE: Department of Cell Biology, The Scripps Research Institute, La Jolla, California 92037, USA.  
CONTRACT NUMBER: GM 54604 (NIGMS)  
NS 28121 (NINDS)  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Mar 17) 275 (11) 7771-8.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English

Counts 09/770,102

FILE SEGMENT: Priority Journals  
OTHER SOURCE: GENBANK-AF155647  
ENTRY MONTH: 200004  
ENTRY DATE: Entered STN: 20000421  
Last Updated on STN: 20000421  
Entered Medline: 20000412

AB Using the cytoplasmic domain of fibroblast growth factor receptor 1 (FGFR1) as bait in a yeast two-hybrid screen, Grb14 was identified as a FGFR1 binding **partner**. A kinase-inactive mutant of FGFR1 failed to interact with Grb14, indicating that activation of FGFR1 is necessary for binding. Deletion of the C-tail or mutation of both C-tail tyrosine residues of FGFR1 to phenylalanine abolished binding, and deletion of the juxtamembrane domain of the receptor **reduced binding**, suggesting that Grb14 binds to FGFR1 at multiple sites.

Co-immunoprecipitation and *in vitro* **binding assays**

demonstrated that binding of Grb14 to FGFR1 in mammalian cells was dependent on receptor activation by fibroblast growth factor-2 (FGF-2). Deletion of the Src homology 2 (SH2) domain of Grb14 reduced but did not block binding to FGFR1 and eliminated dependence on receptor activation. The SH2 domain alone bound both FGFR1 and platelet-derived growth factor receptor, whereas full-length Grb14 bound only FGFR1, suggesting that regions upstream of the SH2 domain confer specificity for FGFR1. Grb14 was phosphorylated on serine and threonine residues in unstimulated cells, and treatment with FGF-2 enhanced this phosphorylation. Expression of exogenous Grb14 inhibited FGF-2-induced cell proliferation, whereas a point-mutated form of Grb14 incapable of binding to FGFR1 enhanced FGF-2-induced mitogenesis. These data demonstrate an interaction between activated FGFR1 and Grb14 and suggest a role for Grb14 in FGF signaling.

L43 ANSWER 3 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 1999383885 MEDLINE  
DOCUMENT NUMBER: 99383885 PubMed ID: 10452803  
TITLE: A microchip-based enzyme assay for **protein kinase A**.  
AUTHOR: Cohen C B; Chin-Dixon E; Jeong S; Nikiforov T T  
CORPORATE SOURCE: Caliper Technologies Corporation, 605 Fairchild Drive, Mountain View, California 94043, USA.  
SOURCE: ANALYTICAL BIOCHEMISTRY, (1999 Aug 15) 273 (1) 89-97.  
Journal code: 0370535. ISSN: 0003-2697.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199909  
ENTRY DATE: Entered STN: 19991012  
Last Updated on STN: 19991012  
Entered Medline: 19990928

AB A microchip-based enzyme assay for **protein kinase A** is described. The microchips were prepared by standard photolithographic techniques. The assay reagents were placed in wells on the microchips, and electroosmosis was used to transport aliquots of these reagents into the network of etched channels, where the enzymatic reaction takes place. **Protein kinase A** catalyzes the transfer of a phosphate group from ATP to the serine residue of the heptapeptide LeuArgArgAlaSerLeuGly (Kemptide). The outcome of the enzymatic reaction was assessed by performing an on-chip electrophoretic separation of the fluorescently labeled **peptide** substrate and product. All liquid-handling steps were performed by controlling the electroosmotically driven flow from reagent and buffer wells using

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electrical current. On-chip dilutions of the peptide substrate, ATP and H-89, a known **protein kinase A inhibitor**, were performed and the kinetic constants ( $K(m)$ ,  $K(i)$ ) of these compounds were determined. This prototype assay demonstrates the usefulness of the microchips for performing enzymatic assays for which fluorogenic substrates cannot easily be designed.

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L43 ANSWER 4 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 1999339930 MEDLINE  
DOCUMENT NUMBER: 99339930 PubMed ID: 10411623  
TITLE: Site-directed removal of N-glycosylation sites in human gastric lipase.  
AUTHOR: Wicker-Planquart C; Canaan S; Riviere M; Dupuis L  
CORPORATE SOURCE: Laboratoire de Lipolyse Enzymatique, UPR 9025 de l'IFR-1 du CNRS, Marseille, France.  
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1999 Jun) 262 (3) 644-51.  
PUB. COUNTRY: Journal code: 0107600. ISSN: 0014-2956.  
DOCUMENT TYPE: GERMANY: Germany, Federal Republic of  
LANGUAGE: Journal; Article; (JOURNAL ARTICLE)  
FILE SEGMENT: English  
ENTRY MONTH: Priority Journals  
199908  
ENTRY DATE: Entered STN: 19990820  
Last Updated on STN: 19990820  
Entered Medline: 19990806

AB Human gastric lipase (HGL) is a highly **glycosylated protein**, as glycan chains account for about 15% of the molecular mass of the native HGL. Four potential N-glycosylation consensus sites (Asn15, 80, 252 and 308) can be identified from the HGL amino acid sequence. We studied the functional role of the individual N-linked oligosaccharide chains by removing one by one all the N-glycosylation sites, via Ala residue replacement by site-directed mutagenesis of Ser and Thr residues from the consensus sequences Asn-X-Ser/Thr. Mutagenized cDNA constructs were heterologously expressed in the baculovirus/insect cell system. Removal of oligosaccharides either at Asn15, 80 or 252 was found to have no significant influence on the **enzymatic activity measured** in vitro. However, the absence of glycosylation at Asn308, as well as a total deglycosylation, reduced the specific enzymatic activity of recombinant HGL (r-HGL), measured on short- and long-chain triglycerides, to about 50% of normal values. Furthermore, biosynthesis and secretion of r-HGL markedly dropped when all four potential glycosylation sites were mutated. The kinetics of the interfacial adsorption of r-HGL and the completely deglycosylated r-HGL (four-site mutant) were found to be identical when recording the changes with time of the surface pressure either at the air-water interface or in the presence of an egg phosphatidylcholine (PtdCho) monomolecular film spread at various initial surface pressures. This indicates that both recombinant HGLs are identical, as far as recognition of phospholipid film and adsorption on PtdCho are concerned. The N-glycosylation of HGL may contribute to the enzyme stability in the stomach, as under acidic conditions the degradation by pepsin of the unglycosylated r-HGL is increased.

L43 ANSWER 5 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 1998083380 MEDLINE  
DOCUMENT NUMBER: 98083380 PubMed ID: 9421643  
TITLE: Digoxigenin-labeled peptides for the

immunological quantification of intracellular signaling proteins: application to the MAP kinase kinase isoform MEK2.

AUTHOR: Blais C Jr; Drapeau G; Meloche S; Morais R; Adam A  
CORPORATE SOURCE: Universite de Montreal, Quebec, Canada.  
SOURCE: BIOCERNEQUINES, (1997 Dec) 23 (C) 1098-103.  
Journal code: 8306785. ISSN: 0736-6205.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199802  
ENTRY DATE: Entered STN: 19980217  
Last Updated on STN: 19980217  
Entered Medline: 19980203

AB Two competitive enzyme immunoassays using digoxigenin-labeled peptides have been developed for the quantification of the protein kinase MEK2 in cell extracts. Rabbit polyclonal antibodies directed against either the amino-terminal or proline-rich amino acid sequences of MEK2 were used for the immunoconcentration of the protein. Anti-digoxigenin Fab fragments labeled with horseradish peroxidase allowed the detection of the immune complexes. Amino-terminal and proline-rich enzyme immunoassays exhibited a sensitivity level of 63 and 71 fmol/mL, respectively, and displayed a half-maximal saturation value of 1320 and 1780 fmol/mL. The intra- and inter-assay coefficients of variation for both assays assessed at three different concentrations of MEK2 were lower than 6% and 12%, respectively. The amount of MEK2 measured by the two methods demonstrated an excellent correlation with the expression level of the protein detected by immunoblot analyses when tested on different cell lysates.

L43 ANSWER 6 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 1998027801 MEDLINE  
DOCUMENT NUMBER: 98027801 PubMed ID: 9361708  
TITLE: Disaccharidase levels in normal epithelium of the small intestine of rats with iron-deficiency anemia.  
AUTHOR: Fernandes M I; Galvao L C; Bortolozzi M F; Oliveira W P;  
Zucoloto S; Bianchi M L  
CORPORATE SOURCE: Departamento de Puericultura e Pediatria, Faculdade de Medicina de Ribeirao Preto, Universidade de Sao Paulo, Brasil.  
SOURCE: BRAZILIAN JOURNAL OF MEDICAL AND BIOLOGICAL RESEARCH, (1997 Jul) 30 (7) 849-54.  
Journal code: 8112917. ISSN: 0100-879X.  
PUB. COUNTRY: Brazil  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199801  
ENTRY DATE: Entered STN: 19980206  
Last Updated on STN: 19980206  
Entered Medline: 19980126

AB Iron-deficiency anemia is the nutritional deficiency most frequently occurring throughout the world, which manifests as a complex systemic disease involving all cells, affecting enzyme activities and modifying protein synthesis. In view of these considerations, the objective of the present study was to determine the effects of iron-deficiency anemia on disaccharidases and on the epithelial morphokinetics of the jejunal mucosa. Newly weaned male Wistar rats were

divided into 4 groups of 10 animals each: C6w received a standard ration containing 36 mg elemental iron per kg ration for 6 weeks; E6w received an iron-poor ration (5-8 mg/kg ration) for 6 weeks; C10w received an iron-rich ration (36 mg/kg ration) for 10 weeks; E10w received an iron-poor ration for 6 weeks and then an iron-rich ration (36 mg/kg) for an additional 4 weeks. Jejunal fragments were used to measure disaccharidase content and to study cell proliferation. The following results were obtained: 1) a significant reduction ( $P < 0.001$ ) of animal weight, hemoglobin (Hb), serum iron and total iron-binding capacity (TIBC) in group E6w as compared to C6w; reversal of the alterations in Hb, serum iron and TIBC with iron repletion (E10w = C10w); animal weights continued to be significantly different in groups E10w and C10w. 2) Sucrase and maltase levels were unchanged; total and specific lactase levels were significantly lower in group E6w and this reduction was reversed by iron repletion (E10w = C10w). 3) The cell proliferation parameters did not differ between groups. On the basis of these results, we conclude that lactase production was influenced by iron deficiency and that this fact was not related to changes in cell population and proliferation in the intestinal mucosa.

L43 ANSWER 7 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 93354943 MEDLINE  
DOCUMENT NUMBER: 93354943 PubMed ID: 8351284  
TITLE: Effects of cholera and pertussis toxins on prolactin stimulation of lactose synthesis and ornithine decarboxylase activity in mouse mammary gland explants.  
AUTHOR: Koduri P B; Rillema J A  
CORPORATE SOURCE: Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan 48201.  
SOURCE: PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, (1993 Sep) 203 (4) 424-7.  
Journal code: 7505892. ISSN: 0037-9727.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199309  
ENTRY DATE: Entered STN: 19931001  
Last Updated on STN: 20021218  
Entered Medline: 19930910

AB Studies indicate that G proteins are likely involved in the signal transduction pathway for prolactin's stimulation of mitogenesis in Nb2 cells. In the mammary gland, little is known about the possible role of G proteins in the prolactin (PRL) stimulation of milk product synthesis. Therefore, the effects of cholera and pertussis toxin, enzymes that modify G protein activity, were tested on several actions of prolactin on mouse mammary tissue in culture. At concentration of 0.1-0.5 micrograms/ml, cholera toxin stimulated ornithine decarboxylase activity in a dose-response fashion; when tested in concert, cholera toxin and prolactin caused an additive response. Cholera toxin by itself did not affect the rate of lactose synthesis, but at concentrations above 0.5 micrograms/ml, it attenuated the magnitude of the prolactin stimulation of lactose synthesis. Pertussis toxin (0-0.5 micrograms/ml), both by itself and in concert with PRL, had no effect on ornithine decarboxylase activity. At concentrations of 25 ng/ml and above, pertussis toxin inhibited the PRL stimulation of lactose synthesis, whereas at 0.2 and 0.5 micrograms/ml, pertussis toxin abolished the PRL response. These observations suggest that a G protein, but not Gs, may be involved in prolactin's mechanism of signal transduction in the mouse

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mammary gland.

L43 ANSWER 8 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 93213434 MEDLINE  
DOCUMENT NUMBER: 93213434 PubMed ID: 1297332  
TITLE: Mutational analysis of a DNA sequence involved in linking gene expression to the cell cycle.  
AUTHOR: Andrews B J; Moore L  
CORPORATE SOURCE: Department of Molecular and Medical Genetics, University of Toronto, Ont., Canada.  
SOURCE: BIOCHEMISTRY AND CELL BIOLOGY, (1992 Oct-Nov) 70 (10-11) 1073-80.  
PUB. COUNTRY: Journal code: 8606068. ISSN: 0829-8211.  
DOCUMENT TYPE: Canada  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199305  
ENTRY DATE: Entered STN: 19930521  
Last Updated on STN: 19930521  
Entered Medline: 19930506

AB Entry of budding yeast cells into the mitotic cell cycle requires the activity of a conserved regulatory kinase encoded by the CDC28 gene. The kinase is thought to trigger entry into the cell cycle or START, through association with a number of regulatory subunits known as G1 cyclins. A number of genes whose transcription is dependent on CDC28 and thus linked to START are controlled by two transcription factors, SWI4 and SWI6. The genes controlled by SWI4 and SWI6 include two known G1 cyclins (CLN1 and CLN2), a putative new G1 cyclin (HCS26), and the HO gene whose product initiates cell type switching. SWI4 and SWI6 act through a repeated sequence element, SCB (SWI4,6-dependent cell cycle box), found 2-10 times in the upstream regulatory sequences of target genes. We have constructed a library of mutants in the SCB using doped oligonucleotide mutagenesis. All single base pair changes examined compromised the ability of the SCB to activate transcription in vivo. Analysis of the behaviour of the mutant SCBs in an in vitro DNA binding assay shows that the inability to activate transcription can be explained by reduced binding of SWI4 and SWI6 to the mutant SCBs. This analysis, together with a consideration of the SCBs found upstream of known SWI4,6-dependent genes, leads to the proposal of a revised consensus sequence for this important regulatory element.

L43 ANSWER 9 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 92267257 MEDLINE  
DOCUMENT NUMBER: 92267257 PubMed ID: 1316856  
TITLE: Insulin can rapidly increase cell surface insulin binding capacity in rat adipocytes. A novel mechanism related to insulin sensitivity.  
AUTHOR: Eriksson J; Lonnroth P; Smith U  
CORPORATE SOURCE: Department of Medicine II, Sahlgren's Hospital, University of Goteborg, Sweden.  
SOURCE: DIABETES, (1992 Jun) 41 (6) 707-14.  
PUB. COUNTRY: Journal code: 0372763. ISSN: 0012-1797.  
DOCUMENT TYPE: United States  
LANGUAGE: English  
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
ENTRY MONTH: 199206  
ENTRY DATE: Entered STN: 19920710

Last Updated on STN: 20000303  
Entered Medline: 19920619

AB To elucidate the acute effect of insulin on its receptor, rat adipocytes were preincubated with insulin, washed with KCN to inhibit receptor cycling, and **125I-labeled insulin binding** was measured. Preincubating cells from young insulin-sensitive rats with insulin **increased** cell surface **binding** up to approximately fourfold without changing apparent receptor affinity. This effect was rapid ( $t_{1/2}$  less than 5 min) and had a similar dose-response relationship as the effect on glucose transport. It was also energy dependent because preincubation with KCN completely abolished the effect of subsequent insulin exposure. The **increased binding** capacity was not recovered after cell solubilization or in partially purified receptors or isolated plasma membranes. Cells pretreated with insulin were less sensitive to the ability of trypsin to remove cell surface receptors, suggesting a conformational change of the receptors. This was also supported by the finding that the polyclonal binding in insulin-treated but not in control cells. Vanadate mimicked the effect of insulin to **increase** insulin **binding**, whereas concanavalin A, vasopressin, phorbol esters, or the adenosine analogue phenyl isopropyl adenosine was without effect. Insulin-resistant adipocytes from obese rats displayed no **increase** in cell surface **binding** after insulin treatment, despite normal tyrosine kinase activity in response to insulin. Thus, both insulin and vanadate elicit a rapid effect to markedly increase the number of cell surface insulin binding sites in intact rat adipocytes. This appears to occur independently of protein kinase C and the inhibitory GTP binding protein (Gi). Furthermore, the effect of insulin could not be demonstrated in insulin-resistant cells, suggesting that this mechanism may be of importance for the regulation of insulin sensitivity.

L43 ANSWER 10 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 92133733 MEDLINE  
DOCUMENT NUMBER: 92133733 PubMed ID: 1723248  
TITLE: Automated nonisotopic assay for **protein**-tyrosine kinase and **protein**-tyrosine phosphatase activities.  
AUTHOR: Babcock J; Watts J; Aebersold R; Ziltener H J  
CORPORATE SOURCE: Biomedical Research Centre, University of British Columbia, Vancouver, Canada.  
SOURCE: ANALYTICAL BIOCHEMISTRY, (1991 Aug 1) 196 (2) 245-51.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199202  
ENTRY DATE: Entered STN: 19920322  
Last Updated on STN: 19980206  
Entered Medline: 19920228

AB A sensitive, automated, and nonisotopic assay for **protein**-tyrosine kinases and phosphatases has been developed. The assay uses commercially available antiphosphotyrosine monoclonal antibodies and the recently developed particle concentration immunofluorescence **immunoassay** technology. The assay is specific for phosphotyrosine residues, can be performed faster, and is at least 100-fold more sensitive than the current standard filter type radioassay. Myelin basic protein and a synthetic peptide corresponding to the autophosphorylation site of p56lck performed equally well in the

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detection of p56lck kinase activity. Myelin basic protein phosphorylated on tyrosine residues by p56lck was successfully used as substrate in the detection of phosphatase activity and vanadate or molybdate were shown to inhibit the phosphatase activity. The assay is particularly useful for the rapid detection of enzyme activities in column fractions from biochemical procedures steps and also for screening of large numbers of potential inhibitors or activators of protein-tyrosine kinases and phosphatases.

L43 ANSWER 11 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 89216617 MEDLINE  
DOCUMENT NUMBER: 89216617 PubMed ID: 3244116  
TITLE: Species specificity of antibodies to regulatory subunits of cyclic AMP-dependent protein kinases.  
AUTHOR: Maddox A M; Steiner A L; Shenolikar S  
CORPORATE SOURCE: Division of Hematology-Oncology, University of Texas Health Science Center, Houston, Texas.  
CONTRACT NUMBER: AM 28163 (NIADDK)  
SOURCE: SECOND MESSENGERS AND PHOSPHOPROTEINS, (1988) 12 (2-3) 83-94.  
PUB. COUNTRY: Journal code: 9002049. ISSN: 0895-7479.  
DOCUMENT TYPE: United States  
LANGUAGE: Journal; Article; (JOURNAL ARTICLE)  
FILE SEGMENT: English  
ENTRY MONTH: Priority Journals  
ENTRY DATE: 198905  
Entered STN: 19900306  
Last Updated on STN: 19970203  
Entered Medline: 19890530

AB Polyclonal antibodies were generated against regulatory subunits (RI and RII) of type-I and type-II cAMP-dependent protein kinases from rat skeletal muscle. Western immunoblot analyses showed specific cross-reactivity of rat and bovine RI with anti-RI. Similarly, RII from both species was specifically recognized by anti-RII. Quantitative immunoassays, using antisera against proteins from either species, indicated selectivity towards regulatory subunits from the same species. Molecular basis for this selectivity was examined by comparison of peptide maps of 32P-8-azido-cAMP-labelled or autophosphorylated peptides. Detailed analysis of two-dimensional peptide fingerprints demonstrated extensive homology between either RI or RII from the two species. The data suggests that the overall protein-chemical and functional determinants characterizing type-I and type-II regulatory subunits of cyclic AMP dependent protein kinase from different species are substantially similar. However, minor differences in structure, also predicted by amino-acid sequences for RI and RII obtained by molecular cloning, may account for the distinct immunological properties of the proteins from rat and bovine tissues.

L43 ANSWER 12 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 88199575 MEDLINE  
DOCUMENT NUMBER: 88199575 PubMed ID: 2452235  
TITLE: Protein tyrosine kinase activity and its endogenous substrates in rat brain: a subcellular and regional survey.  
AUTHOR: Hirano A A; Greengard P; Huganir R L  
CORPORATE SOURCE: Laboratory of Molecular and Cellular Neuroscience, Rockefeller University, New York, New York.  
CONTRACT NUMBER: GM 07524 (NIGMS)  
NS-21550 (NINDS)

Counts 09/70,102

SOURCE: JOURNAL OF NEUROCHEMISTRY, (1988 May) 50 (5) 1447-55.  
PUB. COUNTRY: Journal code: 2985190R. ISSN: 0022-3042.  
United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198806  
ENTRY DATE: Entered STN: 19900308  
Last Updated on STN: 19970203  
Entered Medline: 19880602

AB The rat CNS contains high levels of tyrosine-specific **protein kinases** that specifically phosphorylate the tyrosine-containing synthetic peptide poly(Glu80,Tyr20). The phosphorylation of this peptide is rapid and occurs with normal Michaelis-Menten kinetics. Using this peptide to assay for enzyme activity, we have measured the **protein tyrosine kinase** activity in homogenates from various regions of rat CNS. A marked regional distribution pattern was observed, with high activity present in cerebellum, hippocampus, olfactory bulb, and pyriform cortex, and low activity in the pons/medulla and spinal cord. The distribution of **protein tyrosine kinase** activity was examined in various subcellular fractions of rat forebrain. The majority of the activity was associated with the particulate fractions, with enrichment in the crude microsomal (P3) and crude synaptic vesicle (LP2) fractions. Moreover, the subcellular distribution of pp60csrc, a well-characterized **protein tyrosine kinase**, was examined by immunoblot analysis using an affinity-purified antibody specific for pp60csrc. The subcellular distribution of pp60csrc paralleled the overall **protein tyrosine kinase** activity. In addition, using an antibody specific for phosphotyrosine, endogenous substrates for **protein tyrosine kinases** were demonstrated on immunoblots of homogenates from the various regions and the subcellular fractions. The immunoblots revealed numerous phosphotyrosine-containing proteins that were present in many of the CNS regions examined and were associated with specific subcellular fractions. The differences in tyrosine-specific **protein kinase** activity, and in phosphotyrosine-containing proteins, observed in various regional areas and subcellular fractions may reflect specific functional roles for **protein tyrosine kinase** activity in mammalian brain.

L43 ANSWER 13 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 86085909 MEDLINE  
DOCUMENT NUMBER: 86085909 PubMed ID: 3001081  
TITLE: Regulation of epidermal growth factor receptor number and phosphorylation by fasting in rat liver.  
AUTHOR: Freidenberg G R; Klein H H; Kladde M P; Cordera R; Olefsky J M  
CONTRACT NUMBER: AM 07494 (NIADDK)  
AM 33650 (NIADDK)  
AM 33651 (NIADDK)  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1986 Jan 15) 261 (2) 752-7.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198602  
ENTRY DATE: Entered STN: 19900321

Last Updated on STN: 20000303

Entered Medline: 19860214

AB The binding of  $^{125}\text{I}$ -epidermal growth factor (EGF) to microsomal membrane preparations from the livers of rats fasted for 72 h or fed control or high carbohydrate diets was examined to determine whether alterations in nutrient intake could affect the EGF receptor system. Fasted rats had 40-50% less membrane binding than did control or carbohydrate-fed rats. Scatchard analysis of the binding data indicated that the **decrease** in EGF **binding** in fasted rats was due to a decrease in receptor number with no change in receptor affinity. Cross-linking of  $^{125}\text{I}$ -EGF to EGF receptors with disuccinimidyl suberate revealed specific binding of a Mr 170,000 protein, which was diminished by approximately 75% in fasting, and a Mr = 150,000 protein, which accounted for 40-50% of the total **labeling** in the control and carbohydrate-fed rats and which was relatively unchanged by fasting. The sum of the **labeling** of the 2 bands was reduced by approximately 40% in fasting and is consistent with the **reduction** in EGF **binding detected** by Scatchard analysis. EGF stimulated a 1.5-3-fold increase in  $^{32}\text{P}$  incorporation into one major protein of 170 kDa in all 3 groups. Basal and EGF-stimulated autophosphorylation of 170 kDa, when normalized for protein, was 75% lower in membranes from fasted animals, compared to those from control or carbohydrate-fed rats. The comparable **reduction** of  $^{125}\text{I}$ -EGF **binding** to, and  $^{32}\text{P}$  incorporation into, the 170-kDa EGF receptor protein suggested that **kinase activity** /receptor was unaffected by fasting. Moreover, EGF receptor **kinase activity** in the 3 groups was comparable for an exogenous substrate, as judged by equal basal and EGF-stimulated phosphorylation of Val15-angiotensin II, when normalized for total EGF-binding capacity. These results suggest that fasting regulates EGF receptor **kinase activity** primarily by regulation of the number of hepatic EGF receptors. The possibility exists that some *in vivo* effects of fasting may be mediated by a reduction in EGF receptor levels.

L43 ANSWER 14 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 85074210 MEDLINE  
DOCUMENT NUMBER: 85074210 PubMed ID: 3965138  
TITLE: Pattern of endogenous lectins in a human epithelial tumor.  
AUTHOR: Gabius H J; Engelhardt R; Cramer F; Batge R; Nagel G A  
SOURCE: CANCER RESEARCH, (1985 Jan) 45 (1) 253-7.  
Journal code: 2984705R. ISSN: 0008-5472.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198501  
ENTRY DATE: Entered STN: 19900320  
Last Updated on STN: 19900320  
Entered Medline: 19850125

AB Salt and detergent extracts of a malignant epithelial tumor, obtained by extraction of acetone powder, were fractionated on different sets of Sepharose columns covalently derivatized with lactose, asialofetuin, melibiose, mannan, fucose, and heparin. Successive elution by chelating reagent and specific sugar resulted in isolation of different  $\text{Ca}^{2+}$ -dependent and  $\text{Ca}^{2+}$ -independent endogenous carbohydrate-binding proteins, as analyzed by gel electrophoresis. It appears from the analysis that certain bands represent newly identified proteins capable of binding to lactose (at Mr 64,000), melibiose (at Mr 28,000), and fucose (at Mr 62,000 and 70,000). Other carbohydrate-binding proteins isolated

from this human tumor have been identified in normal, especially embryonic, tissues of different nonhuman vertebrates. The carbohydrate-binding proteins are assayable as agglutinin with rabbit erythrocytes and show no detectable enzymatic activity. They can thus be defined as lectins. The presence of a complex pattern of endogenous lectins and their biochemical characteristics may contribute to an understanding of intercellular interaction during the complex process of metastatic spread and may furthermore allow a new tool for diagnosis and a lectin-based therapy.

L43 ANSWER 15 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 84104174 MEDLINE  
DOCUMENT NUMBER: 84104174 PubMed ID: 6661210  
TITLE: Importance of albumin binding in the assay for carnitine palmitoyltransferase.  
AUTHOR: McCormick K; Notar-Francesco V J  
CONTRACT NUMBER: AM 01129 (NIADDK)  
SOURCE: BIOCHEMICAL JOURNAL, (1983 Nov 15) 216 (2) 495-8.  
Journal code: 2984726R. ISSN: 0264-6021.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198402  
ENTRY DATE: Entered STN: 19900319  
Last Updated on STN: 19980206  
Entered Medline: 19840214

AB Alterations in the long-chain acyl-CoA binding to albumin in the carnitine palmitoyltransferase (CPT) assay appreciably affect the reaction at commonly used substrate concentrations. Since in the CPT assay the latter are typically well below saturation or Vmax. values, the measured enzyme activity depends on both the absolute quantity of albumin in the CPT assay and any biochemical modification of its binding. The present study verifies the striking dependence of the K0.5 for palmitoyl-CoA on albumin and the misleading 'activation' of the enzyme by compounds that also avidly bind to albumin. In assessing the intracellular physiological relevance of any modifier of CPT, the effects of protein binding in the assay assume particular importance. Indeed, any compound that alters CPT activity may do so, not directly, but as an assay artifact changing the free or unbound substrate concentrations.

L43 ANSWER 16 OF 61 MEDLINE on STN  
ACCESSION NUMBER: 84036412 MEDLINE  
DOCUMENT NUMBER: 84036412 PubMed ID: 6313970  
TITLE: Epidermal growth factor receptor metabolism and protein kinase activity in human A431 cells infected with Snyder-Theilen feline sarcoma virus or harvey or Kirsten murine sarcoma virus.  
AUTHOR: Cooper J A; Scolnick E M; Ozanne B; Hunter T  
CONTRACT NUMBER: CA14195 (NCI)  
CA17096 (NCI)  
CA28485 (NCI)  
+  
SOURCE: JOURNAL OF VIROLOGY, (1983 Dec) 48 (3) 752-64.  
Journal code: 0113724. ISSN: 0022-538X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198312  
ENTRY DATE: Entered STN: 19900319  
Last Updated on STN: 20000303  
Entered Medline: 19831217

AB When human A431 cells, which carry high numbers of epidermal growth factor (EGF) receptors, are exposed to EGF, the total content of phosphotyrosine in cell protein is increased, the EGF receptor becomes phosphorylated at tyrosine, and new phosphotyrosine-containing 36,000- and 81,000-dalton proteins are detected. We examined the properties of A431 cells infected with Snyder-Theilen feline sarcoma virus, whose transforming protein has associated tyrosine protein kinase activity, and Harvey and Kirsten sarcoma viruses, whose transforming proteins do not. In all cases, the infected cells were more rounded and more capable of anchorage-independent growth than the uninfected cells. EGF receptors were assayed functionally by measuring EGF binding and structurally by metabolic labeling and immunoprecipitation. In no case did infection appear to alter the rate of EGF receptor synthesis, but infection reduced EGF receptor stability by about 50% for cloned Harvey sarcoma virus-infected cells and by 80% for cloned feline sarcoma virus-infected cells. The corresponding reductions in EGF binding were 70 and 90%, respectively. The proteins of feline sarcoma virus-infected A431 cells contained an increased amount of phosphotyrosine, and the 36,000- and 81,000-dalton phosphoproteins were detected. The EGF receptor was not detectably phosphorylated at tyrosine, however, unless the cells were exposed to EGF. The Harvey and Kirsten sarcoma virus-infected cells did not exhibit elevated levels of phosphotyrosine either in the total cell proteins or in the EGF receptor, nor were the 36,000- and 81,000-dalton proteins detectable. However, these phosphoproteins were found in the infected cells after EGF treatment. Thus, all of the infected A431 cells exhibited reduced EGF binding and increased degradation of EGF receptors, yet their patterns of protein phosphorylation were distinct from those of EGF-treated A431 cells.

L43 ANSWER 17 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2002:978582 HCPLUS  
DOCUMENT NUMBER: 138:68915  
TITLE: Assay for protein-modifying enzymes using tagged binding partner polypeptides and applications to drug screening  
INVENTOR(S): Craig, Roger  
PATENT ASSIGNEE(S): UK  
SOURCE: U.S. Pat. Appl. Publ., 44 pp.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002197606	A1	20021226	US 2001-770102	20010125
PRIORITY APPLN. INFO.:			US 2000-179283P	P 20000131

AB The invention relates to monitoring of enzymic modification-dependent polypeptide interaction. The invention provides methods and compns. for monitoring activity of protein-modifying enzymes, such as protein kinase, protein phosphatase, or protease, as a function of the

interaction of **modification-dependent** binding partner polypeptides. A binding partner polypeptide comprises a binding domain that is capable of binding to a binding domain of another binding partner polypeptide in a **modification-dependent** manner. Assocn. or dissochn. of the binding partner polypeptides is dependent upon the addn. or removal of a moiety to or from one or both of the binding partner polypeptides or upon proteolytic digestion of one or both of the binding partner polypeptides by a protein-modifying enzyme. One way to quant. measure the effect of a protein-modification enzyme is to label one or both of the **modification-dependent** binding partner polypeptides with fluorescent labels. The interaction between the binding partners can then be followed in soln. using fluorescence resonance energy transfer (FRET) or fluorescence polarization (FP)-based assays, where one or both of the partners involved in the interaction are labeled with a fluorophore. Alternatively, immobilized formats can be used, where one binding partner polypeptide is fixed to a solid surface and the binding of a second, fluorescently labeled partner is detected. The methods of the invention can be used in screening for a candidate modulator of enzymic activity. Exemplary measurement of Src protein kinase and Yersinia phosphatase activities by the modulation of FRET between binding partners labeled with fluorescent coiled-coil heterodimers or fluorescent proteins, and measurement of Src and Yersinia phosphatase using an immobilized assay using binding partners labeled with fluorescent coiled-coil heterodimers or fluorescent proteins.

L43 ANSWER 18 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2000:804940 HCPLUS  
DOCUMENT NUMBER: 134:277548  
TITLE: Comparison of the stress response to cryopreservation in monolayer and three-dimensional human fibroblast cultures: stress proteins, MAP kinases, and growth factor gene expression  
AUTHOR(S): Liu, Kang; Yang, Yujun; Mansbridge, Jonathan  
CORPORATE SOURCE: Advanced Tissue Sciences, Inc., La Jolla, CA, USA  
SOURCE: Tissue Engineering (2000), 6(5), 539-554  
CODEN: TIENFP; ISSN: 1076-3279  
PUBLISHER: Mary Ann Liebert, Inc.  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Stress responses induced in fibroblasts by cryopreservation were compared in suspension or three-dimensional cultures at various times up to 5 days of recovery. Cryopreservation caused an 86% inhibition in [35S]methionine incorporation, with recovery over 2 days to 45% .+- 14% of its original value. Stress proteins, including heat shock protein (hsp) and glucose-regulated proteins (GRP), detected by immunoblotting, responded with transient increases in cellular content (hsp27 and hsp90 in suspension and three-dimensional culture, and hsp70 only in three-dimensional culture), decreases at 24 h (hsp56, hsp70, hsp90, and GRP78 in three-dimensional culture and hsp90 in suspension), or little change (hsp70 in suspension). Polyacrylamide gel electrophoresis of [35S]methionine-labeled proteins showed transient induction of hsp47 within 4 h, and increased synthesis of hsp90 and GRP78 and other unidentified proteins at 24 h, but no change in hsp70. The mitogen-activated protein (MAP) kinase, p38, showed a transient increase after thawing, followed by a peak in extracellular signal-regulated kinase at 24 h. The stress-activated protein kinase (JNK) was not activated. In both stress protein and MAP kinase responses, the three-dimensional cultures showed a more intense response than fibroblasts in suspension. Although some

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responses were related to osmotic and cold stress during freezing, others were unique. Cryopreservation induced mRNA for selected growth factors, including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) A chain, which increased 5- to 20-fold at 48 h returning to basal levels by 120 h. Our results indicate the novel finding that cryopreservation of fibroblasts grown in three-dimensional culture induced a specific cellular stress response including growth factors.

REFERENCE COUNT: 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 19 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2000:771647 HCAPLUS  
DOCUMENT NUMBER: 134:83046  
TITLE: p53 protein oxidation in cultured cells in response to pyrrolidine dithiocarbamate: a novel method for relating the amount of p53 oxidation in vivo to the regulation of p53-responsive genes  
AUTHOR(S): Wu, Hsiao-Huei; Thomas, James A.; Momand, Jamil  
CORPORATE SOURCE: Department of Chemistry and Biochemistry, California State University at Los Angeles, Los Angeles, CA, 90032, USA  
SOURCE: Biochemical Journal (2000), 351(1), 87-93  
CODEN: BIJOAK; ISSN: 0264-6021  
PUBLISHER: Portland Press Ltd.  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB A novel method was developed to det. the oxidn. status of proteins in cultured cells. Methoxy-polyethylene glycolmaleimide MW 2000 (MAL-PEG) was used to covalently tag p53 protein that was oxidized at cysteine residues in cultured cells. Treatment of MCF7 breast cancer cells with pyrrolidine dithiocarbamate (PDTC), a metal chelator, resulted in a min. of 25% oxidn. of p53. The oxidized p53 had an av. of one cysteine residue oxidized per p53 protein mol. The effect of PDTC treatment on downstream components of the p53 signal-transduction pathway was tested. PDTC treatment prevented actinomycin D-mediated up-regulation of two p53 effector gene products, murine double minute clone 2 oncoprotein and p21WAF1/CIP1 (where WAF1 corresponds to wild-type p53-activated fragment 1 and CIP1 corresponds to cyclin-dependent kinase-interacting protein 1). Actinomycin D treatment led to accumulation of p53 protein in the nucleus. However, when cells were simultaneously treated with PDTC and actinomycin D, p53 accumulated in both the nucleus and the cytoplasm. The data indicate that an av. of one cysteine residue per p53 protein mol. is highly sensitive to oxidn. and that p53 can be efficiently oxidized by PDTC in cultured cells. PDTC-mediated oxidn. of p53 correlates with altered p53 subcellular localization and reduced activation of p53 downstream effector genes. The novel method for detecting protein oxidn. detailed in the present study may be used to det. the oxidn. status of specific proteins in cells.  
REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 20 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2000:609013 HCAPLUS  
DOCUMENT NUMBER: 133:205085  
TITLE: High throughput assay for protein modification  
INVENTOR(S): Colyer, John; Craig, Roger Kingdon; Maschio, Antonio; Mezna, Mokdad  
PATENT ASSIGNEE(S): Fluorescence Limited, UK

SOURCE: PCT Int. Appl., 128 pp.  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 6  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000050902	A2	20000831	WO 2000-GB669	20000225
WO 2000050902	A3	20001214		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2003032054	A1	20030213	US 1999-259658	19990226
EP 1163526	A2	20011219	EP 2000-906474	20000225
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: GB 1999-4398 A 19990225  
 WO 2000-GB669 W 20000225

AB This invention relates to a method for analyzing a sample comprising:  
~~immobilizing a polypeptide to a phys. support; contacting the immobilized polypeptide with a test sample which may contain an agent capable of modifying the immobilized polypeptide; contacting the immobilized polypeptide with a binding partner polypeptide, wherein the binding of this partner polypeptide to the immobilized polypeptide is at least partly dependent on the modification state of the immobilized polypeptide; and measuring the assocn. of the binding partner polypeptide to the immobilized polypeptide.~~ Src kinase assays involved immobilized fluorescent natural binding partners that were affected by phosphorylation.

L43 ANSWER 21 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN  
 ACCESSION NUMBER: 2000:507619 HCPLUS  
 DOCUMENT NUMBER: 134:37679  
 TITLE: The VIG9 gene products from the human pathogenic fungi Candida albicans and Candida glabrata encode GDP-mannose pyrophosphorylase  
 AUTHOR(S): Ohta, A.; Chibana, H.; Arisawa, M.; Sudoh, M.  
 CORPORATE SOURCE: Dep. Mycol., Nippon Roche Res. Cent., Kamakura, Kanagawa, 257-8530, Japan  
 SOURCE: Biochimica et Biophysica Acta (2000), 1475(3), 265-272  
 CODEN: BBACAO; ISSN: 0006-3002  
 PUBLISHER: Elsevier Science B.V.  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB The authors have identified two genomic DNA fragments from the human pathogenic fungi, Candida albicans (CaVIG9) and Candida glabrata (CgVIG9) that encode GDP-mannose pyrophosphorylase, a key enzyme for protein glycosylation. The VIG9 homologues of CaVIG9 and CgVIG9 complement an identified protein glycosylation-defective mutation, vig9, of Saccharomyces cerevisiae. The nucleotide sequences of the ORFs, which are 83 and 90%

identical to that of the ScVIG9 protein, resp., showed a predicted gene product homologous to *S. cerevisiae* GDP-mannose pyrophosphorylase. The authors exmd. the enzyme activity of a glutathione S-transferase fusion of each VIG9 gene to synthesize GDP mannose in the cell exts. of a heterologous *Escherichia coli* expression system. The authors also developed a method for **detecting** the **enzyme** **activity** using a non-radioactive substrate that would be applicable to high throughput screening.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 22 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2000:180857 HCAPLUS  
DOCUMENT NUMBER: 132:235891  
TITLE: Method and assay for regulation of T cell proliferation  
INVENTOR(S): Kupfer, Abraham; Kupfer, Hannah; Monks, Colin R. F.  
PATENT ASSIGNEE(S): National Jewish Medical and Research Center, USA  
SOURCE: U.S., 12 pp.  
CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6040152	A	20000321	US 1996-775310	19961231
PRIORITY APPLN. INFO.:			US 1996-775310	19961231

AB The present invention discloses a method to regulate T cell proliferation by regulating **protein kinase** C theta (PKC.theta.) in a T cell. Also disclosed are assays for evaluating the ability of a T cell to proliferate in response to an antigen-specific stimulus, a method to regulate T cell proliferation in a mammal *in vivo*, a method to identify compds. which regulate T cell proliferation, and compds. identified thereby. The method and regulatory compds. are useful for treating autoimmune disease, graft-vs.-host disease and allergic disease.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 23 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1999:96908 HCAPLUS  
DOCUMENT NUMBER: 130:293386  
TITLE: Proteome analysis. II. Protein subcellular redistribution: linking physiology to genomics via the proteome and separation technologies involved  
AUTHOR(S): Patton, Wayne F.  
CORPORATE SOURCE: Biosciences Department, Bioanalytical Assay Development Group, Molecular Probes, Inc., Eugene, OR, 97402-9165, USA  
SOURCE: Journal of Chromatography, B: Biomedical Sciences and Applications (1999), 722(1 + 2), 203-223  
CODEN: JCBBEP; ISSN: 0378-4347  
PUBLISHER: Elsevier Science B.V.  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: English  
AB A review with 138 refs. While annotated two-dimensional (2D) gel electrophoresis databases contain thousands of proteins, they do not represent the entire genome. High-mol.-mass proteins in particular are

conspicuously absent from such databases. Filamin is prototypical of this class of proteins since it is a dimer with relative mol. mass (Mr) of 520 000 contg. at least 240 potential phosphorylation sites. Filamin is not readily sepd. by current 2D procedures, and is difficult to study with respect to cycles of phosphorylation-dephosphorylation. Novel technologies are needed to identify biochem. pathways impinging upon such targets. The success of immunofluorescence microscopy as a research tool can be attributed in part to the fact that proteins redistribute in response to a variety of physiol. stimuli. Comparable quant. methods are required in prctome anal. Three components are necessary for development of an approach that is capable of screening for protein redistribution events: (1) subcellular fractionation, (2) **protein labeling** and (3) data acquisition. An integrated approach is presented that utilizes differential detergent fractionation combined with reversible, luminescent protein stains and anal. imaging for high-throughput anal. of signal transduction events leading to protein subcellular redistribution. The procedure has been successfully implemented to rapidly define key second messenger pathways leading to endothelial cell junctional permeability and to guide in the design of a new family of peptide-based anti-inflammatory drugs.

REFERENCE COUNT: 138 THERE ARE 138 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 24 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1998:720030 HCPLUS  
DOCUMENT NUMBER: 130:49456  
TITLE: A method for *in situ* mitotic spindle binding assay  
AUTHOR(S): Giet, Regis; Prigent, Claude  
CORPORATE SOURCE: CNRS UPR41, Groupe Cycle Cellulaire, Universite de Rennes I, Rennes, 35042, Fr.  
SOURCE: Experimental Cell Research (1998), 244(2), 470-473  
CODEN: ECREAL; ISSN: 0014-4827  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The Xenopus centrosome **protein kinase pEg2**, involved in spindle assembly, binds to microtubules polymd. *in vitro*. We have developed a method to investigate the affinity of purified recombinant pEg2 protein for the cellular mitotic spindle. Briefly, cells grown on coverslips are fixed, permeabilized, and incubated with recombinant pEg2 protein. Localization of the protein is revealed by probing with a specific monoclonal antibody that recognizes recombinant but not endogenous pEg2. Using this method we show that recombinant pEg2 binds to microtubules *in vitro*, while, *in vivo*, pEg2 localized only to the mitotic spindle and not the interphase microtubule network. We also demonstrate that the catalytic activity of pEg2 is not necessary for its binding ability. This technique can be used to analyze the binding of various **tagged proteins** to cellular mitotic spindle. (c) 1998 Academic Press.

REFERENCE COUNT: 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 25 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1998:618942 HCPLUS  
DOCUMENT NUMBER: 129:227299  
TITLE: Enzyme-linked immunosorbent assay for measurement of JNK, ERK, and p38 kinase activities  
AUTHOR(S): Forrer, Patrik; Tamaskovic, Rastislav; Jaussi, Rolf

CORPORATE SOURCE: Institute Medical Radiobiology, Paul Scherrer Institute, Villigen, CH-5232, Switz.  
SOURCE: Biological Chemistry (1998), 379(8/9), 1101-1111  
CODEN: BICHF3; ISSN: 1431-6730  
PUBLISHER: Walter de Gruyter & Co.  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB A rapid ELISA for the **enzyme activity**  
**measurement** of 3 well-known mitogen-activated **protein**  
**(MAP) kinases**, JNK2, ERK2, and p38 is described. The assay involves immobilization of the resp. kinase substrates c-Jun, Elk1, or ATF2 on microtiter plates, addn. of the kinase reaction mixt., and measurement of substrate phosphorylation using phospho-epitope-specific antibodies. This novel procedure represents a marked improvement to conventional radioactive MAP kinase assays in terms of quantification, precision, performance at physiol. ATP concn., high throughput, time consumption, and amenability to automation. In addn. to the std. solid phase assay using plastic-bound protein substrates, the authors developed an alternative soln. phase protocol using sol. protein substrates. By comparing the results of the 2 assays, the authors found that MAP kinases retained much of their substrate specificity in the phosphorylation of immobilized protein substrates. The authors obsd. a strong preference of JNK2 and p38 for the phosphorylation of dimeric over monomeric substrates. The authors characterized the kinase inhibitory activity of olomoucine, staurosporine, and SB 203580 for JNK2, ERK2, and p38. Taken together, this assay could assist in the biochem. characterization of MAP kinases and in identifying potent and specific inhibitors of these enzymes.

L43 ANSWER 26 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1998:474209 HCPLUS  
DOCUMENT NUMBER: 129:119366  
TITLE: Determination of enzyme protein of CK-MB, m-AST, and ChE by immunological methods and survey of its applying values  
AUTHOR(S): Kang, Xixiong; Sun, Butong; Sun, Shuyan; Hou, Wei; Xie, Feng; Rong, Moke; Sun, Rongwu  
CORPORATE SOURCE: Dep. Lab. Diagn., Norman Bethune Univ. Med. Sci., Changchun, Peop. Rep. China  
SOURCE: Rinsho Byori (1998), 46(7), 713-717  
CODEN: RBYOAI; ISSN: 0047-1860  
PUBLISHER: Rinsho Byori Kankokai  
DOCUMENT TYPE: Journal  
LANGUAGE: Japanese  
AB In recent decades, because considerable progress has been made due to rapid developments in basic theory and techniques in mol. biol. and immunol., the detn. of trace enzyme proteins is not difficult. We measured the serum concn. of Creatine kinase-MB (CK-MB) mitochondria aspartate aminotransferase (m-AST) and Cholinesterase (ChE) immunol. and compared these findings with those of an **assay of enzyme activity**. Purifn. of enzyme protein and prepn. of serum antibodies monoclonal antibodies established the immunol. assay methods. Equipment and reagents for enzyme activity test used 7150 Biochem. Analyzer. CK-NAC AST and ChE were produced by Trace kits (Australia). CK-MB and m-AST used immunol. inhibition method. CK-MB m-AST ChE of protein detn. used immunol. turbidimetry. The normal group included 150 cases and the 1990 patient group. Results of the 2 methods did not significantly differ for normal controls, but were significantly different in the patient group. These results demonstrated that the two methods differ, although each may have specific clin. significance. How to

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evaluate these differences needs to be studied further, but immunol. assay uses higher values for clin. diagnosis than **enzyme activity assay**.

L43 ANSWER 27 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1997:801500 HCPLUS  
DOCUMENT NUMBER: 128:151077  
TITLE: Identification of essential histidine residues in UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-T1  
AUTHOR(S): Wragg, Stephanie; Hagen, Fred K.; Tabak, Lawrence A.  
CORPORATE SOURCE: Departments of Dental Research and Biochemistry and Biophysics, School of Medicine and Dentistry, University of Rochester, Rochester, NY, 14642, USA  
SOURCE: Biochemical Journal (1997), 328(1), 193-197  
CODEN: BIJOAK; ISSN: 0264-6021  
PUBLISHER: Portland Press Ltd.  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Polypeptide N-acetylgalactosaminyltransferase (I) catalyzes the initial step of mucin-type O-glycosylation. The activity of bovine I isoenzyme T1 (I-T1) was inhibited by modification with di-Et pyrocarbonate (DEPC). I-T1 activity was partially restored by hydroxylamine treatment, indicating that one of the reactive residues was His. I-T1 was protected against DEPC inactivation when UDP-GalNAc and EPO-G, a peptide pseudosubstrate PPDAAGAAPLR, were simultaneously present, whereas the presence of EPO-G alone did not alter DEPC inactivation. However, inclusion of UDP-GalNAc alone potentiated DEPC-inhibition of the enzyme, suggesting that UDP-GalNAc **binding** changes the accessibility or reactivity of an essential His residue. Deletion of the 1st 56 amino acids (including 1 His residue) yielded a fully active secreted form of bovine I-T1. Each of the 14 remaining His residues in I-T1 were mutated to Ala residues, and the recombinant mutants were recovered from COS7 cells. Mutants H211A and H344A resulted in recombinant proteins with no **detectable enzymic activity**. A significant decrease in the initial rate of GalNAc transfer to the substrate was obsd. with mutants H125A and H341A (1 and 6% of wild-type activity, resp.). Mutation of the remaining 10 His residues yielded mutants that were indistinguishable from the wild-type enzyme. Mutagenesis and SDS-PAGE anal. of all N-glycosylation sequons revealed that residues Asn-95 and Asn-552 were occupied by N-linked sugars in COS7 cells. Ablation of either site did not perturb enzyme biosynthesis or enzyme activity.  
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 28 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1995:812120 HCPLUS  
DOCUMENT NUMBER: 123:223921  
TITLE: Modulation of GLUT1 intrinsic activity in clone 9 cells by inhibition of oxidative phosphorylation  
AUTHOR(S): Shi, Yanwei; Liu, Hongzhi; Vanderburg, Gloria; Samuel, Sam Jayanth; Ismail-Beigi, Faramarz; Jung, Chan Y.  
CORPORATE SOURCE: Dep. Biophysical Sciences, State Univ. New York, Buffalo, NY, 14215, USA  
SOURCE: Journal of Biological Chemistry (1995), 270(37), 21772-8  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Brief (1-2 h) exposure of Clone 9 cells to inhibitors of oxidative phosphorylation such as azide is known to markedly increase glucose uptake. Clone 9 cells express GLUT1 but not GLUT2, -3, and -4, and the azide effect was not accompanied by any increase in cellular or plasma membrane GLUT1 level. To identify the mol. event underlying this apparent increase in GLUT1 intrinsic activity, the authors studied the acute effects of azide on the substrate binding activity of GLUT1 in Clone 9 cells by measuring glucose-sensitive cytochalasin B binding. The glucose-displaceable, cytochalasin B binding activity was barely detectable in membranes isolated from Clone 9 cells under control conditions but was readily detectable after a 60-min incubation of cells in the presence of 5 mM azide, showing a 3-fold increase in binding capacity with no change in binding affinity. Furthermore, the cytochalasin B binding activity of purified human erythrocyte GLUT1 reconstituted in liposomes was significantly reduced in the presence of cytosol derived from azide-treated Clone 9 cells but not in the presence of cytosol from control cells; this effect was heat-labile and abolished by the presence of the peptide corresponding to the GLUT1 COOH-terminal sequence. These results suggest that a cytosolic protein in clone 9 cells binds to GLUT1 at its COOH-terminal domain and inhibits its substrate binding and that azide-induced metabolic alteration releases GLUT1 from this inhibitory interaction. Studying the binding of cytosolic proteins derived from 35S-labeled Clone 9 cells to glutathione S-transferase fusion protein contg. glucose transporter COOH-terminal sequences, the authors identified 28- and 70-kDa proteins that bind specifically to the cytoplasmic domain of GLUT1 and GLUT4 in vitro. The authors also found a 32P-labeled, 85-KDa protein that binds to GLUT4 but not to GLUT1 and only in cytosol derived from azide-treated cells. The roles, if any, of these glucose transporter-binding proteins in the azide-sensitive modulation of GLUT1 substrate binding activity in Clone 9 cells are yet to be detd.

L43 ANSWER 29 OF 61 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1994:650037 HCPLUS  
DOCUMENT NUMBER: 121:250037  
TITLE: A novel method for evaluation of carbohydrate-binding activity: enzyme-linked carbohydrate-binding assay (ELCBA)  
AUTHOR(S): Dolzhenko, M. I.; Lepekhin, E. A.; Berezin, V. A.  
CORPORATE SOURCE: Dep. Biophys. Biochem., State Univ., GDniepropetrovsk, 320625, Ukraine  
SOURCE: Biochemistry and Molecular Biology International (1994), 34(2), 261-71  
CODEN: BMBIES; ISSN: 1039-9712  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB. A highly sensitive method for detection of the carbohydrate-binding activity of proteins is described. The method is based on interactions of carbohydrate-binding proteins, immobilized on a solid phase, with an enzyme-labeled sol. polysaccharide (peroxidase-conjugated glycosaminoglycan's heparin, chondroitin sulfate, or hyaluronic acid). Binding capacity was measured spectrophotometrically after enzymic reaction with chromogenic substrate. The reliability of the assay was tested by use of two heparin-binding proteins: (1) fibronectin (sol.) and (2) heparin-binding protein purified from human brain (water-insol.). Binding of heparin was dependent on metal ions, detergents and urea. The assay is believed to be applicable for the identification and

Counts 09/770,102

characterization of a variety of carbohydrate (glycosaminoglycan)-binding proteins, esp. when traditional methods cannot be applied (e.g., when proteins are water-insol.).

L43 ANSWER 30 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1994:239523 HCAPLUS  
DOCUMENT NUMBER: 120:239523  
TITLE: Reversible immunoprecipitation using histidine- or glutathione S-transferase-tagged staphylococcal protein A  
AUTHOR(S): Poon, Randy Y. C.; Hunt, Tim  
CORPORATE SOURCE: Imperial Cancer Res. Fund, Clare Hall Lab., Hertfordshire, EN6 3LD, UK  
SOURCE: Analytical Biochemistry (1994), 218(1), 26-33  
CODEN: ANBCA2; ISSN: 0003-2697  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The authors have constructed, expressed, and purified hexahistidine- and glutathione S-transferase (GST)-tagged Staphylococcal protein A. The histidine-tagged protein A bound efficiently to iminodiacetic acid (IDA)-Sepharose loaded with Zn<sup>2+</sup>, and the GST-protein A was efficiently retained by glutathione-Sepharose. Both recombinant forms of protein A can be used in the normal way to harvest immune complexes with IgG. Both forms of protein A can be released from the Sepharose matrix by mild procedures. The His6-protein A:antibody:antigen complexes can be released from the matrix with EDTA, and immunoppts. bound to GST-protein A can be released either by elution with glutathione or by digestion with thrombin. The authors tested this method with immunoppts. of the p40M015 protein kinase, and found that they retained their ability to phosphorylate p33cdk2 after elution from the affinity matrixes.

L43 ANSWER 31 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1991:20645 HCAPLUS  
DOCUMENT NUMBER: 114:20645  
TITLE: EIA of protein kinase C isozyme  
INVENTOR(S): Hidaka, Hiroyoshi  
PATENT ASSIGNEE(S): Japan  
SOURCE: Jpn. Kokai Tokkyo Koho, 11 pp.  
CODEN: JKXXAF  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 02138871	A2	19900528	JP 1988-227935	19880912
PRIORITY APPLN. INFO.:			JP 1988-188875	19880728

AB The title EIA uses a monoclonal antibody specific to the isoenzyme and an antibody specific to the catalytic region of protein kinase C and reactive with a 40-mer peptide contg. the catalytic site (sequence given). Thus, connective tissue ext. was placed in a microplate sensitized with monoclonal antibody to the isoenzyme and incubated with peroxidase-labeled anti-protein kinase C monoclonal antibody, followed by the bound peroxidase measurement for protein kinase C detn.

L43 ANSWER 32 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN

Counts 09/770,102

ACCESSION NUMBER: 1990:607228 HCAPLUS  
DOCUMENT NUMBER: 113:207228  
TITLE: Detection and isolation of the NADPH-binding protein  
of the NADPH:O2 oxidoreductase complex of human  
neutrophils  
AUTHOR(S): Green, Terrence R.; Pratt, Katherine L.  
CORPORATE SOURCE: Dep. Biochem. Mol. Biol., Oregon Health Sci. Univ.,  
Portland, OR, 97201, USA  
SOURCE: Journal of Biological Chemistry (1990), 265(31),  
19324-9  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Neutrophils assayed with nitro blue tetrazolium (NBT) exhibit  
intracellular rather than extracellular superoxide-generating activity  
when stimulated with phorbol myristate acetate. Enzyme activity is  
stimulated by anionic detergents, reversibly inhibited by 2',3'-NADPH  
dialdehyde; and present in equal levels in membrane fractions obtained  
from phorbol myristate acetate-stimulated and resting cell suspensions.  
Solubilized membrane shows enzyme activity co-eluting on mol. sieving  
columns with the cytochrome b redox component of the oxidoreductase  
complex. Enzyme activity was resolved free of the cytochrome b component  
following passage of solubilized membrane exts. through QAE-Sephadex anion  
exchange columns. **Enzyme activity measured**  
by the NBT assay appears to be that assocd. with the NADPH binding protein  
of the oxidoreductase complex. When exposed to NBT and NADPH this  
component of the oxidoreductase generate superoxide independent of  
cytochrome b.

L43 ANSWER 33 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1980:563445 HCAPLUS  
DOCUMENT NUMBER: 93:163445  
TITLE: Kinetics of irreversible enzyme inhibition: the  
interpretation of the fractional enzyme activity vs.  
extent of protein modification plot  
AUTHOR(S): Rakitzis, Emmanuel T.  
CORPORATE SOURCE: Med. Sch., Univ. Athens, Athens, 620, Greece  
SOURCE: Journal of Theoretical Biology (1980), 85(3), 553-60  
CODEN: JTBIAP; ISSN: 0022-5193  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The interpretation of the fractional enzyme **activity** vs. extent  
of **protein modification** plot in **enzyme**  
irreversible inhibition studies is currently performed by either: (1) the  
probabilistic treatment of Tsou Chen-Lou (1962) or (2) an intuitive  
approach used by most research workers. Examn. of whether the intuitive  
approach is justified on theor. grounds, it was found that except for some  
cases of strong irreversible **binding** cooperativity, the  
intuitive method is erroneous. Since this plot is the ratio of the  
equation describing protein modification and the equation describing  
enzyme activity loss, an interpretation of the plot is not possible unless  
both of the constituent functions have been analyzed independently. On  
the other hand, it is shown that when the probabilistic treatment is  
applied to cases of irreversible **binding** cooperativity, care  
should be taken to effect corrections appropriate to the case under  
consideration.

L43 ANSWER 34 OF 61 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1980:39733 HCAPLUS

Counts 09/770,102

DOCUMENT NUMBER: 92:39733  
TITLE: Competitive protein binding assay  
INVENTOR(S): Yoshida, Robert A.; Maggio, Edward T.; Zuk, Robert F.  
PATENT ASSIGNEE(S): Syva Co., USA  
SOURCE: Brit. UK Pat. Appl., 21 pp.  
CODEN: RAXXDU  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
GB 2001172	A	19790124	GB 1978-29892	19780714
GB 2001172	B2	19820127		
US 4208479	A	19800617	US 1977-815632	19770714
US 4233401	A	19801111	US 1977-815487	19770714
CA 1102693	A1	19810609	CA 1978-307277	19780712
AU 7838002	A1	19800117	AU 1978-38002	19780713
AU 518002	B2	19810910		
NL 7807607	A	19790116	NL 1978-7607	19780714
JP 54020134	A2	19790215	JP 1978-86009	19780714
JP 63001544	B4	19880113		
CH 648414	A	19850315	CH 1978-7673	19780714
PRIORITY APPLN. INFO.:			US 1977-815487	19770714
			US 1977-815632	19770714

AB A member of an immunol. pair (ligand-receptor) was detd. by a competitive protein binding assay including the ligand or receptor to be detd., enzyme-conjugate, ligand/receptor (antiligand), enzyme inhibitor (antienzyme), and enzyme substrates. The enzyme activity in the assay medium was detd. and comparison with enzyme activity with a known amt. of ligand/receptor allowed quant. detn. of the amt. of ligand/receptor in the sample. E.g., a soln. contg. digoxin, digoxin-glucose 6-phosphate dehydrogenase conjugate, and antidigoxin was incubated for 10 min at 30.degree.. .beta.-NAD was added and the mixt. assayed for 0.5 min (340 nm, 30.degree.) followed by addn. of anti(glucose 6-phosphate dehydrogenase) and assayed at 340 nm (30.degree., 5.5 min). The concn. of digoxin could be detd. over a 10<sup>4</sup> range at concns. as low as 10<sup>-8</sup>-10<sup>-9</sup>M.

L43 ANSWER 35 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 2001:859 BIOSIS  
DOCUMENT NUMBER: PREV200100000859  
TITLE: Mutagenesis analysis of human SM22: Characterization of actin binding.  
AUTHOR(S): Fu, Yiping; Liu, Hong Wei; Forsythe, Sean M.; Kogut, Paul; McConville, John F.; Halayko, Andrew J.; Camoretti-Mercado, Blancá; Solway, Julian (1)  
CORPORATE SOURCE: (1) Dept. of Medicine, University of Chicago, 5841 S. Maryland Ave., Chicago, IL, 60637:  
jsolway@medicinebsd.uchicago.edu USA  
SOURCE: Journal of Applied Physiology, (November, 2000) Vol. 89, No. 5, pp. 1985-1990. print.  
ISSN: 8750-7587.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB SM22 is a 201-amino acid actin-binding protein expressed at high levels in.

smooth muscle cells. It has structural homology to calponin, but how SM22 binds to actin remains unknown. We performed site-directed mutagenesis to generate a series of NH<sub>2</sub>-terminal histidine (His)-tagged mutants of human SM22 in Escherichia coli and used these to analyze the functional importance of potential actin binding domains. Purified full-length recombinant SM22 bound to actin in vitro, as demonstrated by cosedimentation assay. Binding did not vary with calcium concentration. The COOH-terminal domain of SM22 is required for actin affinity, because COOH terminally truncated mutants (SM22-(1-186) and SM22-(1-166)) exhibited markedly reduced cosedimentation with actin, and no actin binding of SM22-(1-151) could be detected. Internal deletion of a putative actin binding site (154-KKAQEHKR-161) partially prevented actin binding, as did point mutation to neutralize either or both pairs of positively charged residues at the ends of this region (KK154LL and/or KR160LL). Internal deletion of amino acids 170-180 or 170-186 also partially or almost completely inhibited actin cosedimentation, respectively. Of the three consensus protein kinase C or casein kinase II phosphorylation sites in SM22, only Ser-181 was readily phosphorylated by protein kinase C in vitro, and such phosphorylation greatly decreased actin binding. Substitution of Ser-181 to aspartic acid (to mimic serine phosphorylation) also reduced actin binding. Immunostains of transiently transfected airway myocytes revealed that full-length NH<sub>2</sub>-terminal FLAG-tagged SM22 colocalizes with actin filaments, whereas FLAG-SM22-(1-151) does not. These data confirm that SM22 binds to actin in vitro and in vivo and, for the first time, demonstrate that multiple regions within the COOH-terminal domain are required for full actin affinity.

L43 ANSWER 36 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 2000:372132 BIOSIS  
DOCUMENT NUMBER: PREV200000372132  
TITLE: Development of high throughput screening assays using fluorescence polarization: Nuclear receptor-ligand-binding and kinase/phosphatase assays.  
AUTHOR(S): Parker, Gregory J. (1); Law, Tong Lin; Lenoch, Francis J.; Bolger, Randall E.  
CORPORATE SOURCE: (1) PanVera Corporation, 545 Science Drive, Madison, WI, 53711 USA  
SOURCE: Journal of Biomolecular Screening, (April, 2000) Vol. 5, No. 2, pp. 77-88. print.  
ISSN: 1087-0571.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB Fluorescence polarization (FP) has been used to develop high throughput screening (HTS) assays for nuclear receptor-ligand displacement and kinase inhibition. FP is a solution-based, homogeneous technique requiring no immobilization or separation of reaction components. The FP-based estrogen receptor (ER) assay is based on the competition of fluorescein-labeled estradiol and estrogen-like compounds for binding to ER. These studies determined the Kd for this interaction to be 3 nM for ERalpha and 2 nM for ERbeta; IC50 values for 17beta-estradiol, tamoxifen, 4-OH-tamoxifen, and diethylstibestrol were determined to be 5.6, 189, 26, and 3.5 nM, respectively. In a screen of 50 lead compounds from a transcriptional activation screen, 21 compounds had IC50 values below 10 muM, with one having an almost 100-fold higher affinity for ERbeta over ERalpha. These data show that an FP-based competitive binding assay can be used to screen diverse compounds with a broad range

of binding affinities for ERs. The FP-based protein-tyrosine kinase (PTK) assay uses fluorescein-labeled phosphopeptides bound to anti-phosphotyrosine antibodies. Phosphopeptides generated by a kinase compete for this binding. In c-Src kinase reactions, polarization decreased with time as reaction products displaced the fluorescein-labeled phosphopeptide from the anti-phosphotyrosine antibodies. The experimentally determined IC<sub>50</sub> of AG 1478 was 400 pM, while Genistein did not inhibit the epidermal growth factor receptor at similar concentrations. Like the FP-based PTK assay, the protein kinase C (PKC) assay utilizes competition: PKC isoforms had different turnover rates for the peptide substrate. The IC<sub>50</sub> for staurosporine was less than 10 nM for all PKC isoforms. Tyrosine phosphatase assays use direct binding rather than competition. Increasing concentrations of T-cell protein-tyrosine phosphatase (TC PTP) increased the rate of dephosphorylation. This change in polarization was dependent on TC PTP and was inhibited by 50 μM Na<sub>3</sub>VO<sub>4</sub>. The IC<sub>50</sub> of Na<sub>3</sub>VO<sub>4</sub> was 4 nM for TC PTP. These data demonstrate that a FP-based assay can detect kinase and phosphatase activity. Homogeneous, fluorescent techniques such as FP are now methods of choice for screening many types of drug targets. New HTS instrumentation and assay methods like these make FP a technology easily incorporated into HTS.

L43 ANSWER 37 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 2000:364618 BIOSIS  
DOCUMENT NUMBER: PREV200000364618  
TITLE: Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease.  
AUTHOR(S): Gong, Cheng-Xin; Lidsky, Theodore; Wegiel, Jerzy; Zuck, Lorinda; Grundke-Iqbali, Inge; Iqbal, Khalid (1)  
CORPORATE SOURCE: (1) New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Rd., Staten Island, NY, 10314 USA  
SOURCE: Journal of Biological Chemistry, (February 25, 2000) Vol. 275, No. 8, pp. 5535-5544. print.  
ISSN: 0021-9258.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB Hyperphosphorylated tau, which is the major protein of the neurofibrillary tangles in Alzheimer's disease brain, is most probably the result of an imbalance of tau kinase and phosphatase activities in the affected neurons. By using metabolically competent rat brain slices as a model, we found that selective inhibition of protein phosphatase 2A by okadaic acid induced an Alzheimer-like hyperphosphorylation and accumulation of tau. The hyperphosphorylated tau had a reduced ability to bind to microtubules and to promote microtubule assembly in vitro. Immunocytochemical staining revealed hyperphosphorylated tau accumulation in pyramidal neurons in cornu ammonis and in neocortical neurons. The topography of these changes recalls the distribution of neurofibrillary tangles in Alzheimer's disease brain. Selective inhibition of protein phosphatase 2B with cyclosporin A did not have any significant effect on tau phosphorylation, accumulation, or function. These studies suggest that protein phosphatase 2A participates in regulation of tau phosphorylation, processing, and function in vivo. A down-regulation of protein phosphatase 2A activity can lead to Alzheimer-like abnormal hyperphosphorylation of tau.

Counts 09/770,102

L43 ANSWER 38 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1999:530396 BIOSIS  
DOCUMENT NUMBER: PREV199900530396  
TITLE: Receptor binding protein amperometric affinity sensor for rapid beta-lactam quantification in milk.  
AUTHOR(S): Setford, S. J. (1); Van Es, R. M.; Blankwater, Y. J.; Kroger, S.  
CORPORATE SOURCE: (1) Cranfield Biotechnology Centre, Cranfield University, Bedfordshire, MK43 0AL UK  
SOURCE: Analytica Chimica Acta, (Oct., 1999) Vol. 398, No. 1, pp. 13-22.  
ISSN: 0003-2670.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB Screen-printed devices, incorporating working electrode immobilised beta-lactam specific receptor **binding protein**, were employed to **measure** penicillin G levels in milk. Quantification was achieved through ELISA-based affinity-assay format coupled to amperometric determination of bound **enzyme** label **activity**. Assay inhibition increased from zero, in the absence of penicillin G in milk, to 33.5 and 77.1% reduction in signal response in the presence of 5 mug kg<sup>-1</sup> and 10 mug kg<sup>-1</sup> penicillin G, respectively. The maximum residue limit of penicillin G in milk for consumption is 5 mug kg<sup>-1</sup>, as defined by the FDA. Coefficient of variation values varied from 4.2-26.4%. The assay incorporates a 2-4 min incubation step, a rapid washing step and 1-2 min measurement step. The receptor binding protein is specific for the major beta-lactam antibiotic types. The assay is simple to perform and requires minimum reagent usage, making it ideal as a field-based screening tool for beta-lactam quantification in milk.

L43 ANSWER 39 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1999:355088 BIOSIS  
DOCUMENT NUMBER: PREV199900355088  
TITLE: High density O-glycosylation on tandem repeat peptide from secretory MUC1 of T47D breast cancer cells.  
AUTHOR(S): Mueller, Stefan; Alving, Kim; Peter-Katalinic, Jasna; Zachara, Natasha; Gooley, Andrew A.; Hanisch, Franz-Georg (1)  
CORPORATE SOURCE: (1) Medical Faculty of the University, Institute of Biochemistry, Joseph-Stelzmann-Strasse 52, 50931, Koeln Germany  
SOURCE: Journal of Biological Chemistry, (June 25, 1999) Vol. 274, No. 26, pp. 18165-18172.  
ISSN: 0021-9258.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB The site-specific O-glycosylation of MUC1 tandem repeat peptides from secretory mucin of T47D breast cancer cells was analyzed. After affinity isolation on immobilized BC3 antibody, MUC1 was partially deglycosylated by enzymatic treatment with alpha-sialidase/beta-galactosidase and fragmented by proteolytic cleavage with the Arg-C-specific endopeptidase clostrypain. The PAP20 glycopeptides were isolated by reversed phase high pressure liquid chromatography and subjected to the structural analyses by quadrupole time-of-flight electrospray ionization mass spectrometry and to the sequencing by Edman degradation. All five positions of the repeat peptide were revealed as O-

glycosylation targets in the tumor cell, including the Thr within the DTR motif. The degree of substitution was estimated to average 4.8 glycans per repeat, which compares to 2.6 glycosylated sites per repeat for the mucin from milk (Mueller, S., Goletz, S., Packer, N., Gooley, A. A., Lawson, A. M., and Hanisch, F.-G. (1997) J. Biol. Chem. 272, 24780-24793). In addition to a modification by glycosylation, the immunodominant DTR motif on T47D-MUC1 is altered by amino acid replacements (PAPGSTAPAAHGVTSAPESR), which were revealed in about 50% of PAP20 peptides. The high incidence of these replacements and their detection also in other cancer cell lines imply that the conserved tandem repeat domain of MUC1 is polymorphic with respect to the peptide sequence.

L43 ANSWER 40 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1999:310045 BIOSIS  
DOCUMENT NUMBER: PREV199900310045  
TITLE: Homo- and heterodimerization of synapsins.  
AUTHOR(S): Hosaka, Masahiro; Sudhof, Thomas C. (1)  
CORPORATE SOURCE: (1) University of Texas Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, TX, 75235-9111 USA  
SOURCE: Journal of Biological Chemistry, (June 11, 1999) Vol. 274, No. 24, pp. 16747-16753.  
ISSN: 0021-9258.

DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB In vertebrates, synapsins constitute a family of synaptic vesicle proteins encoded by three genes. Synapsins contain a central ATP-binding domain, the C-domain, that is highly homologous between synapsins and evolutionarily conserved in invertebrates. The crystal structure of the C-domain from synapsin I revealed that it constitutes a large (>300 amino acids), independently folded domain that forms a tight dimer with or without bound ATP. We now show that the C-domains of all synapsins form homodimers, and that in addition, C-domains from different synapsins associate into heterodimers. This conclusion is based on four findings: 1) in yeast two-hybrid screens with full-length synapsin IIa as a bait, the most frequently isolated prey cDNAs encoded the C-domain of synapsins; 2) quantitative yeast two-hybrid protein-protein binding assays demonstrated pairwise strong interactions between all synapsins; 3) immunoprecipitations from transfected COS cells confirmed that synapsin II heteromultimerizes with synapsins I and III in intact cells, and similar results were obtained with bacterial expression systems; and 4) quantification of the synapsin III level in synapsin I/II double knockout mice showed that the level of synapsin III is decreased by 50%, indicating that heteromultimerization of synapsin III with synapsins I or II occurs in vivo and is required for protein stabilization. These data suggest that synapsins coat the surface of synaptic vesicles as homo- and heterodimers in which the C-domains of the various subunits have distinct regulatory properties and are flanked by variable C-terminal sequences. The data also imply that synapsin III does not compensate for the loss of synapsins I and II in the double knockout mice.

L43 ANSWER 41 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1999:241645 BIOSIS  
DOCUMENT NUMBER: PREV199900241645  
TITLE: Vitamin D-dependent suppression of human atrial natriuretic peptide gene promoter activity requires heterodimer assembly.  
AUTHOR(S): Chen, Songcang; Costa, Claudia H. R. M.; Nakamura, Karl; Ribeiro, Ralff C. J.; Gardner, David G. (1)

CORPORATE SOURCE: (1) Metabolic Research Unit, University of California, San Francisco, CA, 94143-0540 USA  
SOURCE: Journal of Biological Chemistry, (April 16, 1999) Vol. 274, No. 16, pp. 11260-11266.  
ISSN: 0021-9258.

DOCUMENT TYPE: Article  
LANGUAGE: English

SUMMARY LANGUAGE: English

AB Crystallographic structures of the ligand-binding domains for the retinoid X (RXR) and estrogen receptors have identified conserved surface residues that participate in dimer formation. Homologous regions have been identified in the human vitamin D receptor (hVDR). Mutating Lys-386 to Ala (K386A) in hVDR significantly **reduced binding** to glutathione S-transferase-RXRalpha in solution, whereas binding of an I384R/Q385R VDR mutant was almost undetectable. The K386A mutant formed heterodimers with RXRalpha on DR-3 (a direct repeat of AGGTCA spaced by three nucleotides), whereas the I384R/Q385R mutant completely eliminated heterodimer formation. Wild type hVDR effected a 3-fold induction of DR-3-dependent thymidine **kinase-luciferase activity** in cultured neonatal rat atrial myocytes, an effect that was increased to 8-9-fold by cotransfected hRXRalpha. Induction by K386A, in the presence or absence of RXRalpha, was only slightly lower than that seen with wild type VDR. On the other hand, I384R/Q385R alone displayed no stimulatory activity and less than 2-fold induction in the presence of hRXRalpha. Qualitatively similar findings were observed with the negative regulation of the human atrial natriuretic peptide gene promoter by these mutants. Collectively, these studies identify specific amino acids in hVDR that play a critical role in heterodimer formation and subsequent modulation of gene transcription.

L43 ANSWER 42 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1999:212216 BIOSIS

DOCUMENT NUMBER: PREV199900212216

TITLE: Synthesis and secretion of *Providencia rettgeri* and *Escherichia coli* heterodimeric penicillin amidases in *Saccharomyces cerevisiae*.

AUTHOR(S): Ljubijankic, Goran (1); Storici, Francesca; Glisin, Vladimir; Bruschi, Carlo V.

CORPORATE SOURCE: (1) Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, 11001, Beograd Yugoslavia

SOURCE: Gene (Amsterdam), (March 4, 1999) Vol. 228, No. 1-2, pp. 225-232.

ISSN: 0378-1119.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The *Providencia rettgeri* and *Escherichia coli* pac genes encoding heterodimeric penicillin G amidases (PAC) were successfully expressed in *Saccharomyces cerevisiae*. Furthermore, these recombinant enzymes are secreted from the yeast cell into the medium which is in contrast to bacterial hosts, where the enzymes are retained in the periplasm. Contrary to the *P. rettgeri* PAC-encoding gene, the *E. coli* pac is poorly expressed in yeast. The highest yield of *P. rettgeri* PAC was obtained with a multi-copy plasmid, resulting in of 1500 units per liter. This yield is higher by an order of magnitude than that obtained in the best recombinant bacterial expression system. The recombinant *P. rettgeri* enzyme is only partially and selectively O-glycosylated. Only every sixth or seventh alpha-subunit is glycosylated, while the beta-subunit is not glycosylated at all. N-Glycosylation has not been detected.

L43 ANSWER 43 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1999:174829 BIOSIS  
DOCUMENT NUMBER: PREV199900174829  
TITLE: Mechanically induced c-fos expression is mediated by cAMP  
in MC3T3-E1 osteoblasts.  
AUTHOR(S): Fitzgerald, Jamie; Hughes-Fulford, Millie (1)  
CORPORATE SOURCE: (1) Laboratory Cell Growth, Department Medicine, Veterans  
Affairs Medical Center, Mail code 151F, 4150 Clement St.,  
San Francisco, CA 94121 USA  
SOURCE: FASEB Journal, (March, 1999) Vol. 13, No. 3, pp. 553-557.  
ISSN: 0892-6638.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
AB In serum-deprived MC3T3-E1 osteoblasts, mechanical stimulation caused by  
mild (287 X g) centrifugation induced a 10-fold increase in mRNA levels of  
the proto-oncogene, c-fos. Induction of c-fos was abolished by the  
cAMP-dependent protein kinase inhibitor H-89,  
suggesting that the transient c-fos mRNA increase is mediated by cAMP.  
Down-regulation of protein kinase C (PKC) activity by  
chronic TPA treatment failed to significantly reduce c-fos induction,  
suggesting that TPA-sensitive isoforms of PKC are not responsible for  
c-fos upregulation. In addition, 287 X g centrifugation increased  
intracellular prostaglandin E2 (PGE2) levels 2.8-fold ( $P < 0.005$ ). Since we  
have previously shown that prostaglandin E2 (PGE2) can induce c-fos  
expression via a cAMP-mediated mechanism, we asked whether the increase in  
c-fos mRNA was due to centrifugation-induced PGE2 release. Pretreatment  
with the cyclooxygenase inhibitors indomethacin and flurbiprofen did not  
hinder the early induction of c-fos by mechanical stimulation. We conclude  
that c-fos expression induced by mild mechanical loading is dependent  
primarily on cAMP, not PKC, and initial induction of c-fos is not  
necessarily dependent on the action of newly synthesized PGE2.

L43 ANSWER 44 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1999:118519 BIOSIS  
DOCUMENT NUMBER: PREV199900118519  
TITLE: Alternative function of a protein kinase homology domain in  
2',5'-oligoadenylate dependent RNase L.  
AUTHOR(S): Dong, Beihua; Silverman, Robert H. (1)  
CORPORATE SOURCE: (1) Dep. Cancer Biol., NN10, The Lerner Res. Inst.,  
Cleveland Clin. Found., 9500 Euclid Ave., Cleveland, OH  
44195 USA  
SOURCE: Nucleic Acids Research, (Jan. 15, 1999) Vol. 27, No. 2, pp.  
439-445.  
ISSN: 0305-1048.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
AB RNase L is the 2',5'-oligoadenylate (2-5A)-dependent endoribonuclease that  
functions in interferon action and apoptosis. One of the intriguing,  
albeit unexplained, features of RNase L is its significant homology to  
protein kinases. Despite the homology, however, no protein kinase  
activity was detected during activation and RNA cleavage reactions  
with human RNase L. Similarly, the kinase plus ribonuclease domains of  
RNase L produced no detectable protein kinase activity  
in contrast to the phosphorylation obtained with homologous domains of the  
related kinase and endoribonuclease, yeast IRE1p. In addition, neither ATP  
nor pA(2'p5A)3 was hydrolyzed by RNase L. To further investigate the  
function of the kinase homology in RNase L, the conserved lysine at  
residue 392 in protein kinase-like domain II was replaced with an arginine

residue. The resulting mutant, RNase LK392R showed >100-fold decreases in 2-5A-dependent ribonuclease activity without reducing 2-5A- or RNA-binding activities. The greatly reduced activity of RNase LK392R was correlated to a defect in the ability of RNase L to dimerize. These results demonstrate a critical role for lysine 392 in the activation and dimerization of RNase L, thus suggesting that these two activities are intimately linked.

L43 ANSWER 45 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1998:478076 BIOSIS  
DOCUMENT NUMBER: PREV199800478076  
TITLE: Cystatin F is a glycosylated human low molecular weight cysteine proteinase inhibitor.  
AUTHOR(S): Ni, Jian; Fernandez, Marcia Alvarez; Danielsson, Lena; Chillakuru, Rajeev A.; Zhang, Junli; Grubb, Anders; Su, Jeffrey; Geiltz, Reiner; Abrahamson, Magnus (1)  
CORPORATE SOURCE: (1) Dep. Clinical Chem., Inst. Lab. Med., Lund Univ. Hosp., S-221 85 Lund Sweden  
SOURCE: Journal of Biological Chemistry, (Sept. 18, 1998) Vol. 273, No. 38, pp. 24797-24804.  
ISSN: 0021-9258.

DOCUMENT TYPE: Article  
LANGUAGE: English

AB A previously undescribed human member of the cystatin superfamily called cystatin F has been identified by expressed sequence tag sequencing in human cDNA libraries. A full-length cDNA clone was obtained from a library made from mRNA of CD34-depleted cord blood cells. The sequence of the cDNA contained an open reading frame encoding a putative 19-residue signal peptide and a mature protein of 126 amino acids with two disulfide bridges and enzyme-binding motifs homologous to those of Family 2 cystatins. Unlike other human cystatins, cystatin F has 2 additional Cys residues, indicating the presence of an extra disulfide bridge stabilizing the N-terminal region of the molecule. Recombinant cystatin F was produced in a baculovirus expression system and characterized. The mature recombinant protein processed by insect cells had an N-terminal segment 7 residues longer than that of cystatin C and displayed reversible inhibition of papain and cathepsin L ( $K_i = 1.1$  and 0.31 nM, respectively), but not cathepsin B. Like cystatin E/M, cystatin F is a glycoprotein, carrying two N-linked carbohydrate chains at positions 36 and 88. An immunoassay for quantification of cystatin F showed that blood contains low levels of the inhibitor (0.9 ng/ml). Six B cell lines in culture secreted barely detectable amounts of cystatin F, but several T cell lines and especially one myeloid cell line secreted significant amounts of the inhibitor. Northern blot analysis revealed that the cystatin F gene is primarily expressed in peripheral blood cells and spleen. Tissue expression clearly different from that of the ubiquitous inhibitor, cystatin C, was also indicated by a high incidence of cystatin F clones in cDNA libraries from dendritic and T cells, but no clones identified by expressed sequence tag sequencing in several B cell libraries and in >600 libraries from other human tissues and cells.

L43 ANSWER 46 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1993:498537 BIOSIS  
DOCUMENT NUMBER: PREV199396122544  
TITLE: The differential effects of protein kinase C activators and inhibitors on rat anterior pituitary hormone release.  
AUTHOR(S): Thomson, Fiona J.; Johnson, Melanie S.; Mitchell, Rory (1); Wolbers, W. Bart; Ison, Angela J.; Macewan, David J.

Counts 09/770,102

CORPORATE SOURCE: (1) MRC Brain Metabolism Unit, University Dep. Pharmacol.,  
1 George Square, Edinburgh EH8 9JZ UK

SOURCE: Molecular and Cellular Endocrinology, (1993) Vol. 94, No.  
2, pp. 223-234.

ISSN: 0303-7207.

DOCUMENT TYPE: Article

LANGUAGE: English

AB We investigated the possibility that various **protein kinase C** (PKC) activators and inhibitors may differentially affect luteinizing hormone (LH) and growth hormone (GH) release from rat anterior pituitary tissue, incubated in vitro. Activators of PKC induced LH release with the following order of potency: mezerein > phorbol 12,13-dibutyrate (PDBu). Mezerein and PDBu were equipotent on GH release. A range of PKC inhibitors (including compounds highly selective for PKC) potently and completely inhibited PKC activator-induced LH and GH release. Chelerythrine and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7) were less potent inhibitors of PDBu-induced GH release than of LH release. A component of PDBu- and mezerein-induced LH release was inhibited by H7 with high potency, but a second H7-insensitive component was detected. Mezerein- and PDBu-induced GH release consisted of an H7-resistant component only. When the regulatory domain of PKCs from different sources was investigated by displacement of (3H)PDBu binding, the affinity for mezerein was 3-5-fold greater than that for PDBu at PKCs from cerebral cortex, lung and alpha and beta isoforms extensively purified from brain. Anterior pituitary PKCs were unusual in showing closely matched affinity for mezerein and PDBu, reminiscent of their equivalent potency on GH release. In order to investigate the potency of the catalytic domain inhibitor H7 on PKCs from different sources, **enzyme activity assays** were carried out on partially purified cytosolic PKCs from midbrain and anterior pituitary and on extensively purified PKC-alpha and PKC beta. The Ca-2+-independent component of PDBu-induced (phosphatidylserine-dependent) activity from anterior pituitary alone showed unusually low potency of inhibition by H7 but was potently inhibited by staurosporine and Ro 31-8220. In contrast, the Ca-2+-dependent PKC activity in anterior pituitary was inhibited by H7, staurosporine and Ro-31-8220 with high potency as in all other preparations. These results are consistent with the presence and active role in secretion of pharmacologically distinct forms of PKC (or PKC-like kinases) in rat anterior pituitary cells.

L43 ANSWER 47 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1992:409127 BIOSIS

DOCUMENT NUMBER: BA94:72327

TITLE: UBIQUITIN-RAS PEPTIDE EXTENSIONS AS SUBSTRATES FOR FARNESYL-PROTEIN TRANSFERASE AND CARBOXYMETHYLTRANSFERASE.

AUTHOR(S): YOO Y; WATTS S; RECHSTEINER M

CORPORATE SOURCE: DEP. BIOCHEMISTRY, UNIV. UTAH SCH. MED., SALT LAKE CITY,  
UTAH 84132, USA.

SOURCE: BIOCHEM J, (1992) 285 (1), 55-60.  
CODEN: BIJOAK. ISSN: 0306-3275.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB Using oligonucleotide-mediated 'loop-in' mutagenesis strategies in M13, a heat-inducible ubiquitin (Ub) gene was extended by sequences coding for the C-terminal 11 amino acids of Ha-RAS. The resulting gene was transformed into AR13 and production of the Ub-peptide extension was induced by heat treatment. After one-step purification, the fusion protein (Ub-cRAS) was used as a substrate for farnesyl-protein transferase. Ub-cRAS was farnesylated on incubation in Xenopus egg extract or rabbit

Counts 09/770,102

reticulocyte lysate. In contrast, when serine was substituted for the last cysteine in the RAS extension, transfer of the [<sup>3</sup>H]farnesyl group, from [<sup>3</sup>H] farnesyl pyrophosphate to the modified Ub-cRAS was not observed. Farnesylation of Ub-cRAS permitted us to develop an easy membrane-binding assay for farnesyl-protein transferase enzyme activity. Using this assay, we partially purified the enzyme from rabbit reticulocyte lysate. We also detected methylation of the farnesylated Ub-cRAS terminus in Xenopus egg extract.

L43 ANSWER 48 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1987:273780 BIOSIS

DOCUMENT NUMBER: BA84:14819

TITLE: SEQUENCE DEPENDENCE OF DROSOPHILA TOPOISOMERASE II IN PLASMID RELAXATION AND DNA BINDING.

AUTHOR(S): SANDER M; HSIEH T-S; UDVARDY A; SCHEDL P

CORPORATE SOURCE: DUKE UNIV. MED. CENTER, DEP. BIOCHEMISTRY, DURHAM, N.C. 27710.

SOURCE: J MOL BIOL, (1987) 194 (2), 219-230.  
CODEN: JMOBAK. ISSN: 0022-2836.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB The sequence dependence of Drosophila topoisomerase II supercoil relaxation and binding activities has been examined. The DNA substrates used in binding experiments were two fragments from Drosophila heat shock locus 87A7. One of these DNA fragments includes the coding region for the heat shock protein hsp70, and the other includes the intergenic non-coding region that separates two divergently transcribed copies of the hsp70 gene at the locus. The intergenic region was previously shown to have a much higher density of topoisomerase cleavage sites than the hsp70 coding region. Competition nitrocellulose filter binding assays demonstrate a preferential binding of the intergene fragment, and that binding specificity increases with increasing ionic strength. Dissociation kinetics indicate a greater binding specificity increases with increasing ionic strength. Dissociation kinetics indicate a greater kinetic stability of topoisomerase II complexes with the intergene DNA fragment. To study topoisomerase II relaxation activity, we used supercoiled plasmids that contained the same fragments from locus 87A7 cloned as inserts. The relative relaxation rates of the two plasmids were determined under several conditions of ionic strength, and when the plasmid substrates were included in separate reactions or when they were mixed in a single reaction. The relaxation properties of these two plasmids can be explained by a coincidence of high-affinity binding sites, strong cleavage sites, and sites used during the catalysis of strand passage events by topoisomerase II. Sequence dependence of topoisomerase II catalytic activity may therefore parallel the sequence dependence of DNA cleavage by this enzyme.

L43 ANSWER 49 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1981:265358 BIOSIS

DOCUMENT NUMBER: BA72:50342

TITLE: DETECTION OF CORONARY DISEASE PATIENTS AT HIGH RISK FOR RECURRENT MYO CARDIAL INFARCTION BY Elevated PLASMA INACTIVE CREATINE KINASE B PROTEIN LEVELS.

AUTHOR(S): BURNAM M H; CROUCH M A; CHEW C Y C; CARNEGIE W; HECHT H; SINGH B N

CORPORATE SOURCE: CARDIOL SEC. 691/111E, WADSWORTH VA HOSP., WILSHIRE AND SAWTELLE BLVDS., LOS ANGELES, CA 90073.

Counts 09/770,102

SOURCE: AM HEART J, (1981) 101 (5), 561-569.  
CODEN: AHJOA2. ISSN: 0002-8703.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English  
AB The diagnostic and prognostic significance of plasma inactive creatine kinase B protein (CK-Bi) levels measured by radioimmunoassay was determined in various ischemic myocardial syndromes. In 120 stable angina patients free of pain at time of blood sampling, mean CK-Bi level was 114 .+- .42 .mu.g eq/ml; 195 .mu.g eq/ml (95% confidence interval) represented upper limit of normal. In 7 coronary artery disease (CAD) patients atrial pacing-induced ischemia was not associated with increased coronary sinus CK-Bi. Of 201 consecutive patients with suspected acute infarction (AMI), 45 developed ECG criteria of transmural AMI with concomitant increased plasma CK-Bi levels (498 .+- .133, range 372-718 .mu.g eq/ml). Elevated CK-Bi levels in evolving transmural AMI were detected before raised CK enzyme activity. Elevated plasma CK-Bi levels occurred in acute pericarditis and in unstable angina. In the 84 patients not developing ECG changes or elevated plasma CK activity, their plasma CK-Bi levels were normal and no coronary events occurred in the next 6 mo. The remaining 55 patients had nontransmural AMI, with 15 having elevated plasma CK and CK-Bi levels, of whom 6 developed re-AMI in the next 3 mo. In the other 40 nontransmural AMI patients, plasma CK-Bi levels (350 .+- .65 .mu.g eq/ml, range 228-445) increased significantly without associated CK activity rise and 24 developed re-AMI (3 fatal) in the next 6 mo. Plasma CK-Bi protein radioimmunoassay measurement apparently provides a sensitive means for detecting myocardial necrosis or inflammation and elevated plasma CK-Bi levels in coronary disease patients during myocardial ischemic pain may afford identification of a CAD clinical subset at high risk of subsequent AMI.

L43 ANSWER 50 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1981:60200 BIOSIS  
DOCUMENT NUMBER: BR20:60200  
TITLE: SELECTED PROBLEMS AND PROSPECTS OF HIGH SPEED CELL ANALYSIS.  
AUTHOR(S): SCHLAMMADINGER J; GASPAR R JR  
CORPORATE SOURCE: NAGYERDEI KRT. 98., DEBRECEN 4012, HUNG.  
SOURCE: Acta Biol. Acad. Sci. Hung., (1979 (RECD 1981)) 30 (4), 283-302.  
CODEN: ABAHUAU. ISSN: 0001-5288.  
FILE SEGMENT: BR; OLD  
LANGUAGE: English

L43 ANSWER 51 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1980:170195 BIOSIS  
DOCUMENT NUMBER: BA69:45191  
TITLE: CALCIUM AND CYCLIC NUCLEOTIDE DEPENDENT REGULATORY MECHANISMS DURING DEVELOPMENT OF CHICK EMBRYO SKELETAL MUSCLE.  
AUTHOR(S): LE PEUCH C J; FERRAZ C; WALSH M P; DEMAILLE J G; FISCHER E H  
CORPORATE SOURCE: CENT. RECH. BIOCHIM. MACROMOL.-CNRS, BP 5051, 34033 MONTPELLIER CEDEX, FR.  
SOURCE: BIOCHEMISTRY, (1979) 18 (24), 5267-5273.  
CODEN: BICHAW. ISSN: 0006-2960.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English  
AB Late prenatal and early postnatal development of skeletal muscle

regulatory systems was studied in chick embryos from 7 days before to 7 days after hatching. The following protein concentrations or **enzyme activities** were **measured** daily in pectoralis and in leg muscle extracts: parvalbumin, calmodulin (the heat-stable ubiquitous Ca-dependent regulator), Ca-calmodulin-dependent myosin L chain kinase, cyclic(c)AMP dependent and independent **protein kinases**, and the heat-stable **protein kinase** inhibitor. The appearance of adult contractile properties did not correlate with variations in cAMP-dependent **protein kinases** or the **protein kinase** inhibitor, which are already present at day -7 and continue to fluctuate around the same level. Muscle development is accompanied by a decrease of cAMP-independent **protein kinase** activity, which becomes minimal at days +1 to +3, and of the calmodulin content after day +3. These changes may be ascribed to the decrease in the percentage of proliferating cells. The synthesis of Ca-modulated myosin L chain kinase and parvalbumin occurs around the time of hatching, together with that of the sarcoplasmic reticulum Ca<sup>2+</sup>-Mg<sup>2+</sup>-ATPase. Myosin L chain kinase activity, initially low, increases rapidly from day -2 to reach a maximum at day +3 to +4. Parvalbumin, measured by a sensitive radioimmunoassay, is almost absent from all types of muscle until day -2. Active synthesis 1st begins in leg muscles and then in pectoralis muscle several days later (day +4) and at a much lower rate. cAMP-dependent **protein kinase** (and its inhibitor) and calmodulin, the ubiquitous regulatory proteins which mediate the effects of cAMP and Ca<sup>2+</sup> ions, respectively, are synthesized early in embryonic development. Fast muscle differentiation, which involves the switch off of slow-twitch muscle myosin and the withdrawal of multiple innervation, is more closely correlated with the late synthesis of the elements of the Ca cycle, namely, the sarcoplasmic reticulum Ca<sup>2+</sup> pump, the Ca-dependent myosin L chain kinase, and the soluble relaxing factor, parvalbumin.

L43 ANSWER 52 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1980:21308 BIOSIS  
DOCUMENT NUMBER: BR18:21308  
TITLE: ADVANCES IN CYCLIC NUCLEOTIDE RESEARCH VOL. 10. CURRENT METHODOLOGY.  
AUTHOR(S): BROOKER G; GREENGARD P; ROBINSON G A  
SOURCE: BROOKER, G., P. GREENGARD AND G. A. ROBINSON (ED.).  
ADVANCES IN CYCLIC NUCLEOTIDE RESEARCH, VOL. 10. CURRENT METHODOLOGY. XI+259P. RAVEN PRESS: NEW YORK, N.Y., USA.  
ILLUS, (1979) 0 (0), XI+259P.  
CODEN: ACNRCW. ISSN: 0084-5930. ISBN: 0-89004-265-9.  
DOCUMENT TYPE: Book  
FILE SEGMENT: BR; OLD  
LANGUAGE: English

L43 ANSWER 53 OF 61 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN  
ACCESSION NUMBER: 1978:221578 BIOSIS  
DOCUMENT NUMBER: BA66:34075  
TITLE: EFFECT OF THYMOSIN AND LIPO POLY SACCHARIDE ON MURINE LYMPHOCYTE CYCLIC AMP.  
AUTHOR(S): NAYLOR P H; CAMP C E; PHILLIPS A C; THURMAN G B; GOLDSTEIN A L  
CORPORATE SOURCE: DIV. BIOCHEM, DEP. HUM. BIOL., CHEM. GENET., GALVESTON, TEX. 77550, USA.  
SOURCE: J IMMUNOL METHODS, 20 1978, 143-154.  
CODEN: JIMMBG. ISSN: 0022-1759.  
FILE SEGMENT: BA; OLD

Counts 09/770,102

LANGUAGE: English

AB The effects of thymosin and lipopolysaccharide (LPS) on cyclic[*c*]AMP levels in lymphocytes were evaluated using 3 independent assays which included adenine prelabeling, **protein kinase** binding and radioimmunoassay. All 3 assays were sensitive and accurate in assessing relative changes in lymphocytes after incubation in vitro with various agents. The assays confirmed that basal and stimulated levels of cAMP depended on the origin of the lymphocyte population. Each of the 3 techniques demonstrated that pyrogen-free bovine thymosin fraction 5 did not elevate thymocyte cAMP levels. LPS significantly elevated cAMP levels in spleen and thymus lymphocytes. Assays for measuring the activity of thymic extracts in which the intracellular levels of cyclic nucleotides are a criterion for activity are only valid if the preparations are not contaminated with endotoxins.

L43 ANSWER 54 OF 61 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
ACCESSION NUMBER: 97222151 EMBASE

DOCUMENT NUMBER: 1997222151

TITLE: Phosphorylation of Na,K-ATPase by **protein kinase** C at Ser18 occurs in intact cells but does not result in direct inhibition of ATP hydrolysis.

AUTHOR: Feschenko M.S.; Sreadner K.J.

CORPORATE SOURCE: K.J. Sreadner, Massachusetts General Hospital, 149 13th St., Charlestown, MA 02129, United States.

sreadne@helix.mgh.harvard.edu

SOURCE: Journal of Biological Chemistry, (1997) 272/28 (17726-17733).

Refs: 41

ISSN: 0021-9258 CODEN: JBCHA3

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Na,K-ATPase activity has been demonstrated to be regulated by a variety of hormones in different tissues. It is known to be directly phosphorylated on its *alpha*-subunit, but the functional effects of **protein kinases** remain controversial. We have developed a sensitive, antibody-based assay for detection of the level of phosphorylation of the *alpha*.1-isoform of rat Na,K-ATPase at the serine residue that is most readily phosphorylated by **protein kinase** C (PKC) in vitro, Ser18. By stimulation of endogenous PKC and inhibition of phosphatase activity, it was possible to consistently obtain a very high stoichiometry of phosphorylation (close to 0.9) in several types of intact cells. This demonstrates the accessibility and competency of the site for endogenous phosphorylation. The cells used were derived from rat (NRK 52E, C6, L6, and primary cultures of cerebellar granule cells, representing epithelial cells, glia, muscle cells, and neurons). In the presence of the phosphatase inhibitor calyculin A, full phosphorylation was preserved during subsequent assays of enzyme activity in vitro. Assay of the hydrolysis of ATP in NRK and C6 cells, however, indicated that there was no significant effect of phosphorylation on the V(max) of the Na,K-ATPase or on the apparent affinity for Na+. Any regulatory effect of PKC on sodium pump activity thus must be lost upon disruption or permeabilization of the cells and is not a direct consequence of enzyme alteration by covalent phosphorylation of Ser18.

L43 ANSWER 55 OF 61 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
ACCESSION NUMBER: 97094218 EMBASE

Counts 09/770,102

DOCUMENT NUMBER: 1997094218  
TITLE: Biochemical characterization of the **protein**  
tyrosine **kinase** homology domain of the ErbB3  
(HER3) receptor protein.  
AUTHOR: Sierke S.L.; Cheng K.; Kim H.-H.; Koland J.G.  
CORPORATE SOURCE: J.G. Koland, Department of Pharmacology, University Iowa  
College of Medicine, Iowa City, IA 52242, United States  
SOURCE: Biochemical Journal, (1997) 322/3 (757-763).  
Refs: 46  
ISSN: 0264-6021 CODEN: BIJOAK  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The putative **protein** tyrosine **kinase** domain (TKD) of the ErbB3 (HER3) receptor protein was generated as a histidine-tagged recombinant **protein** (hisTKD-B3) and characterized enzymologically. CD spectroscopy indicated that the hisTKD-B3 protein assumed a native conformation with a secondary structure similar to that of the epidermal growth factor (EGF) receptor TKD. However, when compared with the EGF receptor-derived protein, hisTKD-B3 exhibited negligible intrinsic **protein** tyrosine **kinase** activity. Immune complex kinase assays of full-length ErbB3 proteins also yielded no evidence of catalytic activity. A fluorescence assay previously used to characterize the nucleotide-binding properties of the EGF receptor indicated that the ErbB3 protein was unable to bind nucleotide. The hisTKD-B3 protein was subsequently found to be an excellent substrate for the EGF receptor **protein** tyrosine **kinase**, which suggested that in vivo phosphorylation of ErbB3 in response to EGF could be attributed to a direct cross-phosphorylation by the EGF receptor **protein** tyrosine **kinase**.

L43 ANSWER 56 OF 61 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
ACCESSION NUMBER: 93320742 EMBASE  
DOCUMENT NUMBER: 1993320742  
TITLE: Identification of an inducible 85-kDa nuclear  
**protein kinase**.  
AUTHOR: Rachie N.A.; Seger R.; Valentine M.A.; Ostrowski J.;  
Bomsztyk K.  
CORPORATE SOURCE: Dept. of Medicine, University of Washington, Seattle, WA  
98195, United States  
SOURCE: Journal of Biological Chemistry, (1993) 268/29  
(22143-22149).  
ISSN: 0021-9258 CODEN: JBCHA3  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB To identify inducible **protein kinases** localized exclusively in the nucleus, nuclear and cytosolic extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted to an Immobilon-P membrane. After denaturation-renaturation, the membranes were incubated in phosphorylation buffer containing [.gamma.-32P]ATP. Autoradiographs of the membranes revealed an 85-kDa 32P-labeled band; the intensity of this band was transiently increased in nuclear but not in cytosolic extracts from interleukin-1.alpha.-treated cells. Incorporation of 32P label

into a blotted **protein** band suggested the presence of an interleukin-1. $\alpha$ -responsive 85-kDa nuclear **protein kinase**. Fractionation of nuclear extracts by Mono Q failed to separate the kinase activity from the substrate, indicating that the 85-kDa band identified on the Immobilon-P membrane represents a **protein kinase** that undergoes autophosphorylation. Phosphoamino acid analysis of the 85-kDa band showed that this enzyme is a serine/threonine kinase. Purified pp90(RSK) could not be identified by the denaturation-renaturation method, indicating that the 85-kDa kinase identified here is not pp90(RSK). This observation, nuclear but not cytoplasmic localization, and the fact that antibodies to known **protein kinases** failed to recognize it suggest that the enzyme identified here is a novel **protein kinase**.

L43 ANSWER 57 OF 61 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN  
ACCESSION NUMBER: 91324479 EMBASE  
DOCUMENT NUMBER: 1991324479  
TITLE: Determining the extent of **labeling** for tetramethylrhodamine **protein** conjugates.  
AUTHOR: Meadows D.L.; Shafer J.S.; Schultz J.S.  
CORPORATE SOURCE: Allergan Pharmaceuticals, 2525 Dupont Drive, Irvine, CA 92715, United States  
SOURCE: Journal of Immunological Methods, (1991) 143/2 (263-272).  
ISSN: 0022-1759 CODEN: JIMMBG  
COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB A new, relatively simple, spectrophotometric technique has been developed which is useful for accurately determining the extent of chromophore **labeling** of **proteins**. Often the absorbance spectra and extinction coefficients of dye/protein conjugates are strongly affected by changes in the chromophore microenvironment that may occur at high dye/protein ratios. In the method being presented, the microenvironment effects have been significantly reduced by denaturing the dye/protein complex in 6 M guanidine hydrochloride prior to making the necessary spectrophotometric measurements. With this approach, extinction coefficients were obtained under native and denatured conditions for tetramethyl-rhodamine isothiocyanate (TRITC) when bound to a model protein receptor, the sugar binding protein concanavalin A (ConA). The extinction coefficients used for TRITC/ConA conjugates under native and denaturing conditions were  $6.52 \times 10^4$  M<sup>-1</sup> cm<sup>-1</sup> and  $6.96 \times 10^4$  M<sup>-1</sup> cm<sup>-1</sup>, respectively. These values were obtained from a model dye complex formed between TRITC and .epsilon.-amino-n-caproic acid which closely resembles the side chain of lysine residues. Additional cye/ConA conjugates were prepared with tetramethylrhodamine succinimidyl ester (RHS) and eosin isothiocyanate (EITC), and the effects of microenvironment changes on these conjugates were examined. Extinction coefficients for these dyes in native and denaturing conditions, as a function of the degree of labeling, were not appreciably different indicating that changes in the microenvironment did not have a significant affect on the spectral properties of these two dyes. In summary, with this new approach it is quite easy to accurately determine the dye/protein ratio for TRITC conjugates. Also, it is expected that RHS would be a better dye than TRITC for protein conjugation because more accurate values for dye/protein ratios can be obtained under native conditions.

Counts 09/770,102

L43 ANSWER 58 OF 61 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
ACCESSION NUMBER: 2001:33373 SCISEARCH  
THE GENUINE ARTICLE: 387HP  
TITLE: Metastasis-associated protein Mts1 (S100A4) inhibits CK2-mediated phosphorylation and self-assembly of the heavy chain of nonmuscle myosin  
AUTHOR: Kriajevska M; Bronstein I B; Scott D J; Tarabykina S; Fischer-Larsen M; Issinger O G; Lukanidin E (Reprint)  
CORPORATE SOURCE: Danish Canc Soc, Inst Canc Biol, Dept Mol Canc Biol, Strandblvd 49, 4-3, DK-2100 Copenhagen, Denmark (Reprint); Danish Canc Soc, Inst Canc Biol, Dept Mol Canc Biol, DK-2100 Copenhagen, Denmark; Univ York, Dept Chem, York Struct Biol Lab, York YO10 5DD, N Yorkshire, England; Univ York, Dept Biol, York YO10 5DD, N Yorkshire, England; Univ So Denmark, Dept Biochem & Mol Biol, DK-5230 Odense, Denmark  
COUNTRY OF AUTHOR: Denmark; England  
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR CELL RESEARCH, (20 DEC 2000) Vol. 1498, No. 2-3, Sp. iss. SI, pp. 252-263.  
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.  
ISSN: 0167-4889.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 40

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A role for EF-hand calcium-binding protein Mts1 (S100A4) in the phosphorylation and the assembly of myosin filaments was studied. The nonmuscle myosin molecules form bipolar filaments, which interact with actin filaments to produce a contractile force. Phosphorylation of the myosin plays a regulatory role in the myosin assembly. In the presence of calcium, Mts1 binds at the C-terminal end of the myosin heavy chain close to the site of phosphorylation by protein kinase CK2 (Ser1944). In the present study, we have shown that interaction of Mts1 with the human platelet myosin or C-terminal fragment of the myosin heavy chain inhibits phosphorylation of the myosin heavy chain by protein kinase CK2 in vitro. Mts1 might also bind directly the beta subunit of protein kinase CK2, thereby modifying the enzyme activity. Our results indicate that myosin oligomers were disassembled in the presence of Mts1. The short C-terminal fragment of the myosin heavy chain was totally soluble in the presence of an equimolar amount of Mts1 at low ionic conditions (50 mM NaCl). Depolymerization was found to be calcium-dependent and could be blocked by EGTA. Our data suggest that Mts1 can increase myosin solubility and therefore suppress its assembly. (C) 2000 Elsevier Science B.V. All rights reserved.

L43 ANSWER 59 OF 61 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
ACCESSION NUMBER: 94:123164 SCISEARCH  
THE GENUINE ARTICLE: M2503  
TITLE: IDENTIFICATION OF A TPA-RESPONSIVE ELEMENT MEDIATING PREFERENTIAL TRANSACTIVATION OF THE GALANIN GENE PROMOTER IN CHROMAFFIN CELLS  
AUTHOR: ANOUAR Y (Reprint); MACARTHUR L; COHEN J; IACANGELO A L; EIDEN L E  
CORPORATE SOURCE: NIMH, CELL BIOL LAB, MOLEC NEUROSCI SECT, BLDG 36, RM 3A-17, BETHESDA, MD, 20892 (Reprint)  
COUNTRY OF AUTHOR: USA  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (04 MAR 1994) Vol. 269, No. 9, pp. 6823-6831.

DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 53

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The gene encoding the neuropeptide galanin is upregulated by second messenger signal transduction pathways in bovine chromaffin cells. To identify its transcriptional regulatory elements, 5'-flanking sequences of the galanin gene were transiently transfected into primary cultures of bovine chromaffin cells within reporter gene constructs. Multiple regions of the galanin 5' flank seem to be necessary for basal activity. The most promoter-proximal of these regions contains a sequence (TGACG) -66 to -62 nucleotides upstream from the transcriptional start site which mediates stimulation by 12-O-tetradecanoylphorbol- 13 acetate (TPA), as demonstrated by site-directed mutagenesis and cis-activation experiments. This cis-regulatory element mediates preferential TPA stimulation of transcription from the galanin promoter in chromaffin cells compared with bovine endothelial or HeLa cells. DNA-**protein binding assays** indicate that an oligonucleotide that includes the galanin TPA-responsive element (GTRE) binds specifically to proteins from nuclear extracts of chromaffin cells. TPA treatment persistently increases this binding activity in chromaffin but not in endothelial cells. Mutation of the galanin promoter within the -66 to -62 region renders it unresponsive to transcriptional stimulation by TPA, and a correspondingly mutated oligonucleotide fails to bind chromaffin cell nuclear proteins in a gel-shift assay. Chromaffin cell nuclear extracts also contain proteins that bind consensus TPA-responsive (TRE) and cyclic AMP-responsive (CRE) elements. GTRE, TRE, and CRE oligonucleotides all compete more efficiently for **protein** binding to their **labeled** congeners than for **protein** binding to either of the other labeled oligonucleotides, suggesting that the GTRE, TRE, and CRE oligo nucleotides each bind unique as well as common proteins, likely to be members of the Jun/Fos and cAMP-responsive element-binding protein/activating transcription factors (CREB/ATF) families of transcription factors, in chromaffin cells.

L43 ANSWER 60 OF 61 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
ACCESSION NUMBER: 92:426708 SCISEARCH  
THE GENUINE ARTICLE: JC924  
TITLE: UBIQUITIN-RAS PEPTIDE EXTENSIONS AS SUBSTRATES FOR FARNESYL-PROTEIN TRANSFERASE AND CARBOXYMETHYLTRANSFERASE  
AUTHOR: YOO Y (Reprint); WATTS S; RECHSTEINER M  
CORPORATE SOURCE: UNIV UTAH, SCH MED, DEPT BIOCHEM, SALT LAKE CITY, UT, 84132 (Reprint)  
COUNTRY OF AUTHOR: USA  
SOURCE: BIOCHEMICAL JOURNAL, (01 JUL 1992) Vol. 285, Part 1, pp. 55-60.  
ISSN: 0264-6021.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 32

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Using oligonucleotide-mediated 'loop-in' mutagenesis strategies in M13, a heat-inducible ubiquitin (Ub) gene was extended by sequences coding for the C-terminal 11 amino acids of Ha-RAS. The resulting gene was transformed into AR13 and production of the Ub-peptide extension was induced by heat treatment. After one-step purification, the fusion protein

(Ub-cRAS) was used as a substrate for farnesyl-protein transferase. Ub-cRAS was farnesylated on incubation in Xenopus egg extract or rabbit reticulocyte lysate. In contrast, when serine was substituted for the last cysteine in the RAS extension, transfer of the [H-3]farnesyl group from [H-3] farnesyl pyrophosphate to the modified Ub-cRAS was not observed. Farnesylation of Ub-cRAS permitted us to develop an easy membrane-binding assay for farnesyl-protein transferase enzyme activity. Using this assay, we partially purified the enzyme from rabbit reticulocyte lysate. We also detected methylation of the farnesylated Ub-cRAS terminus in Xenopus egg extract.

L43 ANSWER 61 OF 61 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.

(2003) on STN

ACCESSION NUMBER: 97:38086 AGRICOLA  
DOCUMENT NUMBER: IND20566915  
TITLE: Changes in calcium-dependent protein kinase activity during in vitro tuberization in potato.  
AUTHOR(S): MacIntosh, G.C.; Ulloa, R.M.; Raices, M.; Tellez-Inon, M.T.  
CORPORATE SOURCE: Universidad de Buenos Aires, Buenos Aires, Argentina.  
SOURCE: Plant physiology, Dec 1996. Vol. 112, No. 4. p. 1541-1550  
Publisher: Rockville, MD : American Society of Plant Physiologists, 1926-  
CODEN: PLPHAY; ISSN: 0032-0889  
NOTE: Includes references  
PUB. COUNTRY: Maryland; United States  
DOCUMENT TYPE: Article; Conference  
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension  
LANGUAGE: English  
AB A soluble Ca<sup>2+</sup>-dependent protein kinase (CDPK) was purified to homogeneity in potato (*Solanum tuberosum L.*) plants. Potato CDPK was strictly dependent on Ca<sup>2+</sup> (one-half maximal activation 0.6 micromolar) and phosphorylated a wide diversity of substrates, in which Syntide 2 was the best phosphate acceptor (Michaelis constant = 30 micromolar). The kinase was inhibited by Ca<sup>2+</sup>-chelating agents, phenotiazine derivatives, and N-(6-aminoethyl)-5-chloro-1 naphthalenesulfonamide (one-half maximal inhibition = 0.25 micromolar). Polyclonal antibodies directed against the regulatory region of the soybean CDPK recognized a 53-kD polypeptide. In an autophosphorylation assay, this same band was strongly labeled with [ $\gamma$ -32P]ATP in the presence of Ca<sup>2+</sup>. CDPK activity was high in nontuberized plants, but increased 2.5-fold at the onset of tuber development and was reduced to one-half of its original activity when the tuber had completed formation. In the early stages of tuberization, Ca<sup>2+</sup>-dependent phosphorylation of endogenous targets (specific bands of 68, 51, and 46 kD) was observed. These polypeptides were not labeled in nontuberizing plants or in completely formed tubers, indicating that this phosphorylation is a stage-specific event. In addition, dephosphorylation of specific polypeptides was detected in tuberizing plants, suggesting the involvement of a phosphatase. Preincubation of crude extracts with phosphatase inhibitors rendered a 100% increase in CDPK activity.